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ULTRAVIOLET DISINFECTION OF WATER FOR SMALL WATER SUPPLIES

by

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16. ABSTRACT

In this study ultraviolet radiation was considered as an alternative means of disinfection of small drinking water supplies. A major impetus for this study was the large increase in waterborne disease episodes in the United States whose etiologic agent, Giardia lamblia, was found to be highly resistant to conventional chlorination. While the germicidal effect of sunlight has long been known, it has been found that artificial UV with a wavelength of about 260 nm has the maximum microbiocidal action. A common form of artificial UV radiation with a wavelength of 253.7 nm, can be produced by low pressure mercury vapor lamps. The inactivation of microorganisms by UV radiation is based upon photochemical reactions in the DNA which result in errors in the coding system.

Inactivation of microorganisms due to exposure to UV is proportional to the intensity multiplied by the time of exposure. Early attempts to use UV for public water supply treatment beginning in 1910 were not very successful and were abandoned in favor of chlorination. Chlorination can produce by-products which may be carcinogenic to humans and possibly toxic to aquatic life. This fact along with the dramatic increase in giardiasis has spurred a renewed interest in UV for public water supply disinfection. Both field and laboratory studies were used to evaluate the effectivness of UV in reducing the viability of Escherichia coli, Yersinia sp. and Giardia sp. UV sources included commercial UV reactors and an excimer laser.

G. muris was used as a surrogate for G. lamblia so that reliable excystation and a consistent population of infective organisms could be attained throughout the seasons and through the project study period.

G. muris were significantly more resistant to UV than E. coli and more resistant than Yersinia sp. The effectiveness of disinfection was dependent on the amount of UV reaching the organisms, and any hydraulic short-circuiting. The presence of the entrapped air in the commercial UV reactors decreased the efficiency of the reactor.

Color, either natural or added to the test waters decreased the effectiveness of UV disinfection on G. muris. For the range and type of turbidity examined, the shielding effect against bacterial disinfection, note in other studies, was not observed.

Studies on G. muris cysts indicated that storage time and storage temperature affect the viability of the cysts, the rate of decrease in viability approximately doubled with each 10°C increase in temperature above freezing. Below freezing, however, cyst viability was shortened to only hours rather than days for above freezing conditions.

Physical stress produced by pressure and alum addition in water treatment processes appeared to damage and even destroy cysts.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxic Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

Small water supplies, in general, are in need of inexpensive, cost-effective treatment technology to control, through removal or inactivation, biological agents in drinking water. For many small systems in various areas of the United States, an organism of primary concern is Giardia lamblia, a protozoan parasite that has been implicated in a number of waterborne disease outbreaks in recent years. This report describes the results of a study to evaluate the feasibility of ultraviolet disinfection technology for water treatment in small water systems and its efficacy for inactivation of Giardia cysts and bacteria.

Francis T. Mayo, Director
Water Engineering Research
Laboratory

PREFACE

Data on illness associated with drinking water indicates that the 672 outbreaks of waterborne disease reported in the United States between 1946 and 1980 affected more than 150,000 persons (Lippy and Waltrip, 1984).

The identified microbiological agent responsible for a majority of these cases was Giardia lamblia, currently the most common pathogenic intestinal parasite in the U.S. and the leading cause of intestinal disorder in travelers returning from abroad.

It has been known for some time that G. lamblia could be acquired from remote mountain streams flowing from watersheds uninhabited by humans, but which had wild animal populations. Thus, the impression had been generally accepted in the water works profession that giardiasis was only a disease of hikers, hunters, campers, fishermen and skiers who ingested untreated water from these mountain streams. It is now known that the organism infects a wide variety of domestic and wild animals that may act as sources of infection and contamination of water supplies, even those coming from watersheds closed to human access. While the effects of chlorination on the agent G. lamblia are not completely known it is agreed that Giardia cysts are highly resistant to chlorination as commonly practiced in water treatment (Hoff et al., 1979). Thus, the concept that simple chlorination of surface water coming from such closed watersheds renders the water safe for human consumption needs to be re-evaluated.

Another organism of interest is Yersinia enterocolitica, a widespread gastrointestinal bacterium that has been isolated from man and animals with increasing frequency and may be involved in recent waterborne yersiniosis outbreaks.

The final group of organisms of interest in this study was the coliform bacteria group. Common inhabitants of the intestinal tract of man, coliforms are generally considered harmless parasites and are excreted in enormous numbers in the feces each day. Pathogenic organisms may also be discharged by humans who are infected with diseases of the gastrointestinal tract. However, since the pathogens in feces and in polluted waters are difficult to isolate and are present in relatively small numbers, the coliform organisms, which are much more numerous and relatively easy to isolate and enumerate, are used as an indicator group. Generally, the presence of coliform organisms is an indication that pathogens may also be present, and the absence of coliforms is taken as an indication that pathogens are not present, although species such as Giardia can be more resistant than the coliforms.

While chlorine has been the disinfectant of preference for water supplies, ultraviolet (UV) disinfection appears to have promise as an alternative or as a component of the disinfection process especially for small or remote systems. The process, however, needs further research and field testing to ascertain its effectiveness and reliability as a method for assuring the delivery of safe, potable water. Organism response, performance standards, UV reactor geometry, spectral wavelength of the lamp, central versus distributed public UV application, photo reactivation, dark field repair, effects of color and turbidity, costs, and the like are appropriate concerns in developing adequate installations for field use of ultraviolet disinfection. This project evaluated several of these factors in field and laboratory coordinated studies and examined the requirements for providing appropriate field use of UV disinfection in small systems.

ABSTRACT

In this study ultraviolet radiation was considered as an alternative means of disinfection of small drinking water supplies. A major impetus for this study was the large increase in waterborne disease episodes in the United States whose etiologic agent, *Giardia lamblia*, was found to be highly resistant to conventional chlorination. While the germicidal effect of sunlight has long been known it has been found that artificial UV with a wavelength of about 260 nm has the maximum microbiocidal action. A common form of artificial UV radiation with a wavelength of 253.7 nm, can be produced by low pressure mercury vapor lamps. The inactivation of microorganisms by UV radiation is based upon photochemical reactions in the DNA which result in errors in the coding system.

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LIST OF ABBREVIATIONS AND SYMBOLS

A -- absorbance
alk -- alkalinity
APHA -- American Public Health Association
ATCC -- American Type Culture Collection
b -- thickness of fluid layer
°C -- degrees Celsius
cm -- centimeter
cond. -- conductivity
CSTR -- completely stirred treatment reactor
D -- dielectric constant
Δ -- axial dispersion coefficient
δ -- dispersion coefficient
D.O. -- dissolved oxygen
e -- extinction coefficient
EM -- electrophoretic mobility
EMB -- eosin methylene blue
ft -- feet
FMI -- Fluid Metering Inc.
g -- gram
γ -- gravity
gpm -- gallons per minute
HP -- horsepower
hr -- hour
I -- beam intensity
ID -- inside diameter
in. -- inch
K' -- decay coefficient
kV -- kilovolts
L -- liter
Γ -- length of travel, feet
lb -- pounds
MCL -- maximum contaminant level
m -- meter
M -- molar
MDal -- megadaltons
mg -- milligrams
min -- minute
mL -- milliliter
mm -- millimeter
mm^3 -- cubic millimeter
MPN -- most probable number
mV -- millivolt
mW -- milliwatt

NAR	-- nalidixic acid resistant
nm	-- nanometer
no.	-- number
NTU	-- nephelometric turbidity units
Pa	-- pascals
pH	-- negative logarithm of the hydrogen ion activity
psi	-- pounds per square inch
PWS	-- Pure Water Systems, Inc.
R	-- correlation coefficient
R-factor	-- resistance factor in bacteria
ROCCR	-- reverse osmosis concentrate of Cedar River water
rpm	-- revolutions per minute
RTD	-- residence time distribution
S^2	-- variance
sec	-- second
sp.	-- species
τ	-- transmittance
t	-- time
T	-- mean detention time
Ta/Th	-- ratio of actual to theoretical detention time
T. Alk.	-- total alkalinity
T. Hard.	-- total hardness
THM	-- trihalomethane
TTC	-- triphenyltetrazolium chloride
U	-- mean velocity of flow
UTI	-- Ultraviolet Technology, Inc.
UV	-- ultraviolet
V	-- volts
ν	-- viscosity of liquid in poises
YE	-- yeast extract
ZP	-- zeta potential
θ	-- ratio of actual time to mean residence time
μm	-- micrometer
μmhos	-- micromhos
σ	-- standard deviation
λ	-- wavelength

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SECTION 1

INTRODUCTION

ULTRAVIOLET RADIATION

Ultraviolet (UV) radiation is defined as electromagnetic radiation occupying a small portion of the electromagnetic spectrum lying beyond visible light as shown in Figure 1. Electromagnetic radiation is characterized by wavelength λ and frequency υ. Wavelength λ is expressed in terms of nanometers (nm) which is 10^{-9}m. The frequency υ is the number of oscillations per second and is expressed in Hertz. The ultraviolet region of the spectrum has commonly been divided into three subregions: short wavelength (UV-C), ranging from 180 to 280 nm; medium wavelength (UV-B), ranging from 280 to 320 nm, and long wavelength (UV-A), varying from 320 to 380 nm.

Sources of Ultraviolet Light

The sources of UV light may be divided into two classes: natural and artificial. The sun is the most important natural source of UV light although much of its transmitted energy is in the longer wavelength subregion above 295 nm. The radiant energy received from the sun, of course, is responsible for the development and continued existence of life on the earth by photosynthesis. The oxidizing and germ killing effects of sunlight, to a considerable extent, contribute to the conservation of our environment by natural photochemical processes in the atmosphere and by natural ultraviolet purification of surface waters (Schenck, 1979). UV can also be generated artificially by a wide variety of arcs and incandescent lamps. One common form of artificial UV radiation can be generated from special low pressure mercury vapor lamps which produce UV as a result of an electron flow between the electrodes through ionized mercury vapor. These artificial UV radiation sources can supply energy in such relatively high dose rates that, in fractions of a second, a higher degree of disinfection can be accomplished than that by sun irradiation. The low pressure mercury lamps convert input electrical energy into UV energy with a wave length of 253.7 nm.

The UV Germicidal Action Spectrum

While the germicidal effect of sunlight has long been known, certain parts of the UV spectrum have been found to have more

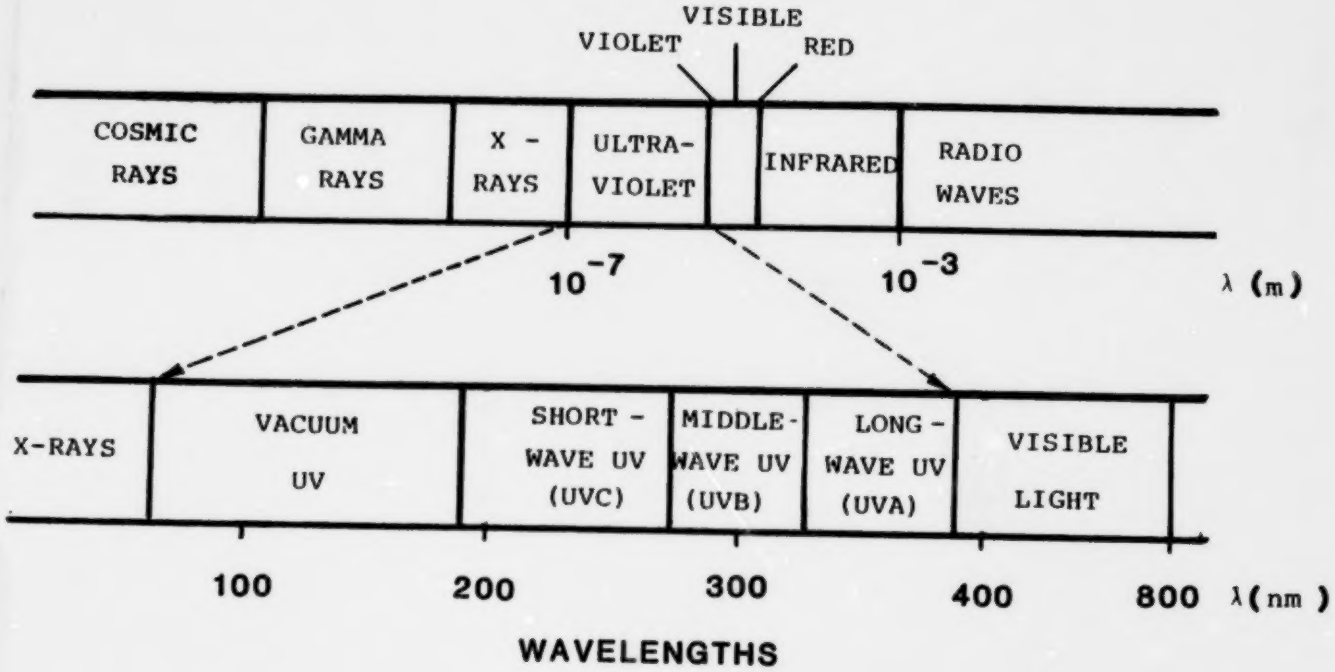

VISIBLE
VIOLET
RED
COSMIC RAYS
GAMMA RAYS
X - RAYS
ULTRA-VIOLET
INFRARED
RADIO WAVES
10^-7
10^-3
λ (m)
X-RAYS
VACUUM UV
SHORT - WAVE UV (UVC)
MIDDLE-WAVE UV (UVB)
LONG - WAVE UV (UVA)
VISIBLE LIGHT
100
200
300
400
800
λ (nm)
WAVELENGTHS

Figure 1. The electromagnetic radiation spectrum with expanded scale of ultraviolet radiation.

pronounced bactericidal action. The wave lengths of UV radiation are from about 4 to 400 nm with the narrow band lying between 200 and 310 nm having the greatest injurious and lethal effects on microorganisms. The maximum microbiocidal action is at about 260 nm for practically all microorganisms. Figures 2 and 3 show the relationship between bactericidal effect and wavelength. As shown in Figure 3 the maximum microbiocidal action of UV is essentially congruent with the UV absorption and photochemical sensitivity of deoxyribonucleic acid (DNA). The inactivation of microorganisms is then essentially based upon photochemical reactions in the DNA which result in errors and faults being introduced into the coding systems. Nature has developed various means of molecular-biological error correction for the protection of the vital DNA and the selectivity of the reactions may be influenced by changes in the organisms in different phases of their life cycle. Thus, photo repair systems may resuscitate a seemingly dead organism by either longer wavelength photon irradiation or dark incubation. Also spores have been found to be radiation resistant. It is fortuitous that the maximum UV sensitivity of microorganisms and the UV emission of the low pressure mercury vapor lamp fit well together. For this reason the nearly monochromatic low pressure mercury lamp has prevailed as the dominant radiation source in research and practical applications (Schenck, 1979).

Principles of UV Disinfection

The germicidal action of UV results from its exposure or direct contact with the organisms and can only be effective if it is absorbed. The lethal effect of UV is due to a photochemical reaction initiated by absorption of a photon by the molecular structure rather than through formation of a toxic substance in the medium (Schenck, 1979, Hoather, 1960). The initial process of disinfection is the transformation of the optical radiation into chemical energy in the absorbing system. The molecule, now in an electronically excited state, may have numerous individual reactions which may either lead back to the regeneration of the molecule with the liberation of heat, to the emission of a photon (luminescence) of lower energy, to a permanent photochemical change or inactivation. The pathway actually followed depends upon environmental factors such as phase, pressure and temperature.

Molecule + photon → Molecule (*) → Molecule + heat
→ Molecule + photon (luminescence)
→ Products (inactivation)

*Electronically excited state.
From Schenck (1979)

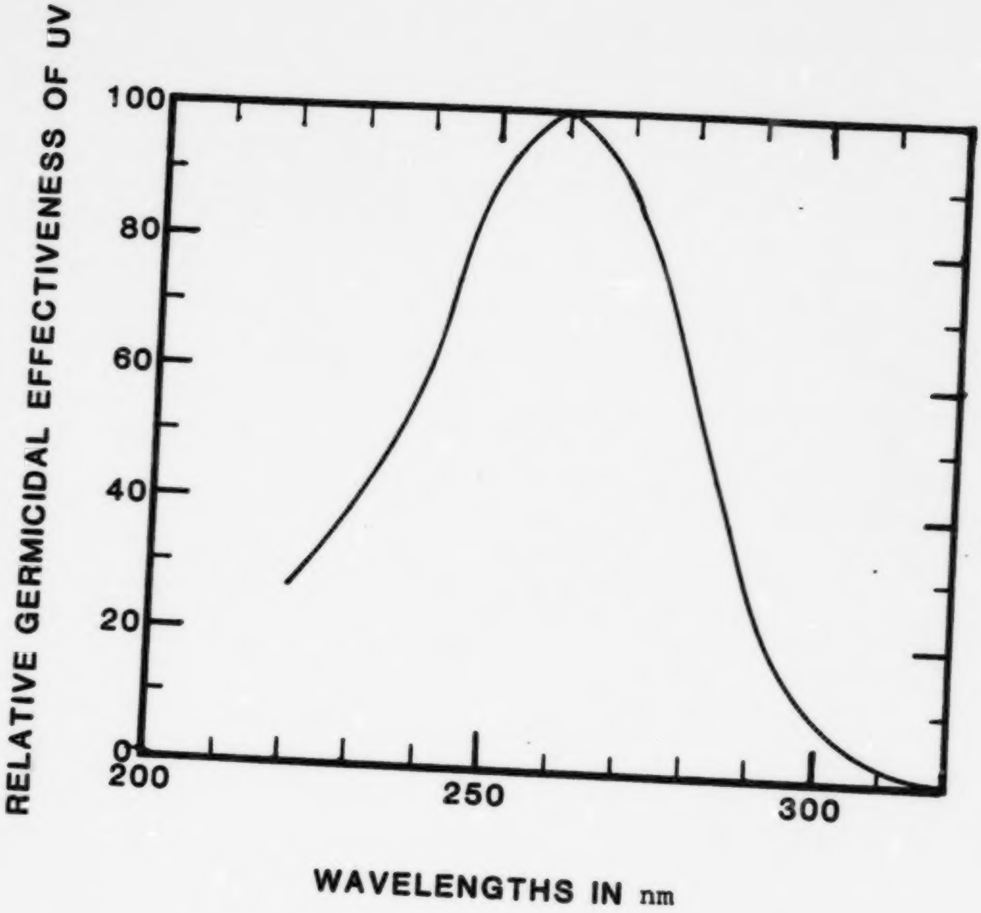
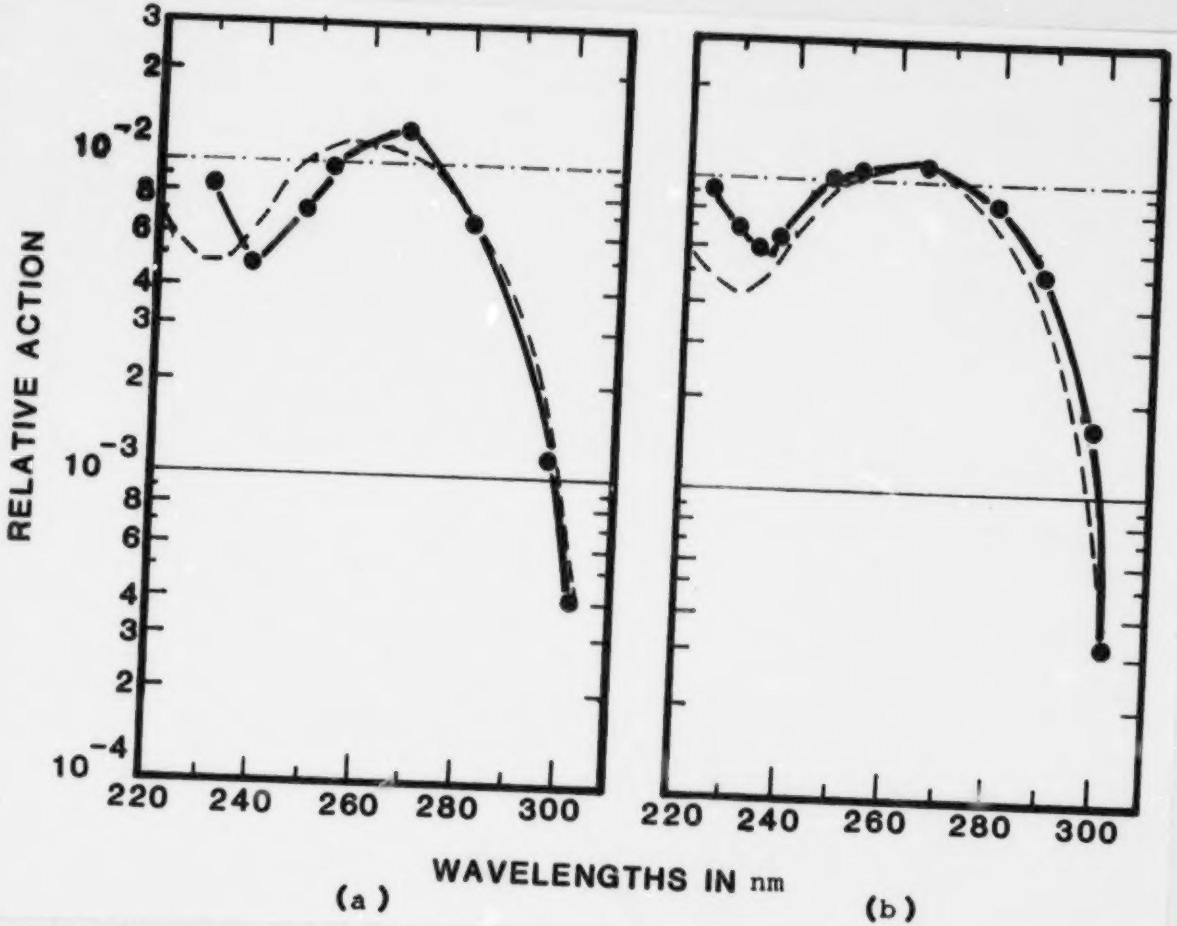


Figure 2. Relative bactericidal effectiveness of ultraviolet radiation.



Action spectra for killing of (a) Escherichia coli and (b) Staphylococcus aureus. The broken lines show the relative absorption of DNA.

Figure 3. Concurrence of DNA absorption of UV energy and germicidal action at different wavelengths.

The inactivation of microorganisms due to exposure to UV can be described as being physical rather than chemical (Hoather, 1960).

The inactivation of microorganisms due to the exposure to UV is proportional to the intensity (mW/cm^2) multiplied by the time of exposure (sec). The product of the irradiation intensity per area and the time is called the UV dose.

Thus, it can be seen that the same exposure can be obtained by using a high intensity for a short time or a low intensity for a proportionally longer period of time (Reddish, 1957, Lawrence and Block, 1968). It should be noted that, with UV radiation, too high a dosage causes no adverse effects except additional costs.

Application of UV to Water Disinfection

The first recorded attempt to use UV for public water supply treatment was made in 1910 at Marseilles, France (Walden and Powell, 1911). Later, four municipalities in the United States adopted UV for water treatment during the period dating from 1916 to 1928 (Baker, 1948, Ellis and Wells, 1941). Other installations of UV equipment were made in various parts of the United States and Central America serving industry, hotels, institutions, and ships, but most were abandoned before 1930 (Baker, 1948). The main reasons given for abandoning the UV method of treatment were: relatively high operating costs, operating and maintenance problems, and the advent of chlorination which was found to be more efficient and reliable. Subsequently, during the decades of the thirties and forties, considerable effort was directed toward the application of UV to the destruction of airborne bacteria and air sanitation (Oda, 1969). With the development of the new tubular germicidal UV lamp, there was renewed interest in UV water disinfection, and considerable experimental data were obtained in numerous studies (Luckiesh and Holladay, 1944, Koller, 1965, Buttolph *et al*., 1953).

However, in spite of the new and more efficient UV equipment, a report in 1948 by the Council on Physical Medicine concluded that UV disinfection was not completely reliable for purification of public water supplies (Council on Physical Medicine, 1948). Subsequent environmental problems and certain limitations associated with chlorination have prompted research into UV for disinfection of potable water and wastewater effluents. Chlorination produces residuals and by-products which may be carcinogenic to humans and which can be toxic to aquatic life in receiving waters. In addition, UV disinfection may be more effective than chlorination in killing viruses (Yip and Konasewich, 1972). The main impetus for this study resulted from the dramatic increase in waterborne disease episodes in the United States since the mid 1960s. Specifically, the widespread occurrence of giardiasis, whose etiologic agent, G. lamblia, has been found to be highly resistant to control by conventional chlorination, has led to renewed interest in UV as an alternative method of water disinfection.

Laser

The acronym "laser" stands for light amplification by stimulated emission of radiation and describes the basic principle first outlined by Albert Einstein. Lasers are devices that amplify or generate radiation by means of the stimulated emission process. One common element of these devices is a medium mode to amplify in a narrow frequency region by population inversion achieved for a pair of energy levels (Lengyel, 1971). Laser light is monochromatic and coherent light at various chosen wavelengths depending upon the gas mixture used for the lasing medium.

SECTION 2

CONCLUSIONS

1. The excystation procedure for Giardia cyst viability was unreliable for G. lamblia cysts and could not be used to provide significant data.

2. To determine cyst viability, G. muris cysts were used as a surrogate of G. lamblia because of their relatively higher percentage of excystability and more consistent reproducibility under laboratory conditions.

3. Unlike chlorine and ozone, UV radiation does not have problems with mixing (mass transfer) in the contactor, but it also does not produce a residual. However, like chlorine and ozone contactors, flow dispersion has a significant impact on UV's biocidal effect.

4. As the UV reactors approached plug flow, greater degrees of disinfection were obtained.

5. Direct measurement of the actual UV dosage in the two commercially available UV contactors was not possible. Instead, it was necessary to rely on actinometry and inactivation of B. subtilis spores to indicate the dosage.

6. The low destruction rate for Giardia cysts reported by previous investigators was primarily due to low UV exposure dose relative to the actual dose required.

7. Giardia cyst inactivation is a function of UV energy absorption and thus dependent on UV light reaching the cyst and the time of exposure. Where commercial UV unit design permits short-circuiting, the time for 100 percent inactivation can be expected to be protracted.

8. The commercial UV disinfection units tested can achieve disinfection of Giardia sp. The design of the units is important in the detention time required for exposure to UV light. For the PWS unit, one percent survival was attained at detention times longer than 60 seconds. In contrast, detention times longer than about 22 seconds were required to reach survival levels less than one percent in the UTI unit.

9. Short-circuiting and collection of air bubbles in commercial UV disinfection units can seriously impair the disinfection capability of the unit.

10. Commercial UV disinfection units could be modified to make more efficient use of the generated UV light and hence increase the feasibility of UV disinfection.

11. From the information attained here, the coherent UV light of the excimer laser appeared to be no more efficient in removal of Giardia cyst viability than UV from mercury vapor tubes in terms of the energy output.

12. Laser equipment available during the experimental period was unreliable and too difficult to operate to be considered for use in field or commercial disinfection systems.

13. For all experimental conditions, color with an absorbance at 254 nm was found to increase cyst survival, e.g. color, caused a decrease in UV disinfection effectiveness.

14. The presence of relatively small particulates, inorganic or organic (5 µm diameter or less), had no discernible effect on UV disinfection of cysts.

15. Turbidity in the form of larger (>5 µm) suspended particulates may provide shielding and protection to the organisms.

16. Virulent Yersinia were less resistant to UV inactivation than Giardia. Hence, if Giardia are removed by UV, Yersinia can be expected to have been destroyed as well.

17. Virulent Yersinia which contained a plasmid demonstrated significantly greater resistance to inactivation by UV than its non-plasmid counterpart.

18. UV was very effective in killing E. coli.

19. The fact that both Yersinia and Giardia cysts were more resistant to UV than E. coli has important implications where the total coliform test is used to monitor microbiological water quality.

20. Size and morphological characteristics of organisms and particles appeared to be a very important factor in shielding from UV radiation.

21. Storage time and storage temperature affect cyst viability, the decrease in viability approximately doubling with each 10°C increase in temperature above freezing. At freezing, however, cyst viability is drastically shortened to hours rather than months and days as it is above freezing.

22. Zeta potentials for G. lamblia cysts were found to be time dependent indicating a change in cyst characteristics with storage.

23. The physical stress produced by pressure and alum addition appeared to damage or even destroy cysts.

SECTION 3

RECOMMENDATIONS

1. The effect of turbidity on disinfection of *Giardia sp*. needs more study. Particularly, the particle size and form and its interference with UV must be investigated.

2. A more practical method of measuring and recording the actual delivered UV dose in the contactor is desperately needed.

3. Further work is needed to improve the accuracy and precision of the *Giardia lamblia* excystation procedure.

4. The effects of storage, temperature and sedimentation on *Giardia sp*. in the water environment need to be ascertained. These may significantly influence the operational mode for treatment of surface water supplies.

5. Pressure and coagulant addition may also have drastic influence on the survival of *Giardia* cysts and thus need to be investigated.

6. Design of UV contactors must eliminate or minimize short circuiting and air entrainment, and optimize reflected radiation to improve biocidal effect.

SECTION 4

OBJECTIVE OF STUDY

The objective of this study was to investigate the effect of ultraviolet (UV) radiation on the survival and viability of Giardia cysts and Yersinia organisms in potable drinking water and upon coliforms as potential indicators of waterborne disease. The germicidal effect of UV radiation has been known for many years and as early as 1910 was used by water authorities for disinfection purposes (Baker, 1948). However, because of the overwhelming acceptance of chlorination as the preferred disinfection method for water supplies, the use of UV disinfection in public water supplies admittedly has been relatively small, in part because UV does not provide a residual in the distribution system. The practice of chlorinating public water supplies began in England around the turn of the century and has spread so that today it is almost considered a routine practice in developed countries. As a result there has been a decrease in the incidence of the traditional waterborne diseases such as typhoid fever, cholera and dysentery. Unfortunately, chlorine also reacts with humic substances that are usually present in natural water, particularly surface water, to form a variety of complex chlorinated hydrocarbon products, known collectively as trihalomethanes (THM). One such compound is chloroform ($CHCl_3$) which may be carcinogenic. The potential for post-chlorination formation of these potentially carcinogenic compounds has led to renewed interest in alternative means of drinking water disinfection. In addition, the indication that Giardia cysts were highly resistant to chlorination has added to the public health concern and the need for a process to mitigate these problems. The benefits of chlorination still are so immense that, in spite of these newly recognized deficiencies, there has been little effort within the water works profession to abandon the practice. However, the concept that simple chlorination of surface water, even those waters coming from watersheds closed to human access, renders the water safe for human consumption needs to be reevaluated. In fact, in three recent outbreaks of waterborne giardiasis (Rome, New York, 1975; Camas, Washington, 1976; and Berlin, New Hampshire, 1977), all of which occurred in mountainous communities with surface water sources with only disinfection by chlorine, it was determined that there was no evidence of sewage contamination of the water. Giardia cysts were recovered from the water in all three instances (Juranek, 1979) and in the

Camas outbreak it was determined that the most likely source of contamination was from infected beavers.

The parameters selected for initial study were those of comparison of UV disinfection sensitivity of coliforms, Giardia and Yersinia, the possible shielding effect of turbidity and color, and the effect of UV wavelength on disinfection sensitivity. The equipment consisted of two commercially available UV water disinfection units, a laboratory batch UV and an excimer laser UV unit to provide coherent UV light at several discrete wavelengths. The studies were carried out in the laboratory and at a field research station located at the Cedar River intake for the City of Seattle water system. It was believed important to evaluate the efficacy of commercially available UV units under field conditions where the natural water was not free of extraneous materials that might react with the disinfectant. Hoff and Geldreich (1981) have previously shown the slowing effect on disinfection rates in water with cell debris and other materials likely to be present in natural water. Among the study components also considered were the reliability of UV units in terms of possible application to small water system operation, energy output, the effect of wavelength, and performance standards of commercially available equipment. It was the purpose then of this study to bring to light some of the characteristics of UV disinfection in a field mode, specifically as related to the biocidal efficiency to Giardia and Yersinia, and to coliforms as indicators of waterborne disease.

SECTION 5

MATERIALS AND METHODS

EXPERIMENTAL PROCEDURES

Assessment of Giardia Viability

Source of *Giardia* Cysts--

In the early stage of this study only *G. lamblia* cysts, supplied mainly by hospitals and pathology laboratories across the State of Washington, were used. However, the supply of *G. lamblia* cysts was closely related to the outdoor recreational activities in the area and hence very seasonal. In addition, the cysts proved difficult to excyst consistently which severely limited the amount of information that could be gathered. Therefore, it was decided to use *Giardia muris* cysts, which according to the information available, appeared at least as resistant as *G. lamblia* (Rice *et al*., 1982).

The justification for this approach is based upon work by Wangsuphachart and Carlson (unpublished). They found the determination of *Giardia* cyst viability was most reliable when using *G. muris* cysts as the indicator species and the excystation procedure developed by Schaefer *et al*. (1980). With *G. muris* cysts, a relatively higher excystability and less fluctuation of the results was obtained than with the other methods, as shown in Figures 4 and 5. Another advantage in using *G. muris* rather than *G. lamblia* cysts was their year around availability from laboratory mice. Until a reliable method to determine the viability of *G. lamblia* is developed, the use of *G. muris* cysts as a surrogate for *G. lamblia* is warranted.

Female Swiss Webster mice were used for cyst propagation. The eight week old mice were infected orally with cysts from a fresh suspension. The incubation period was normally seven days, although on occasions, small amounts of cysts were observed earlier. The infection normally peaked around the tenth day, thereafter decreasing to a rate where only a few cysts were present in the fecal pellets after two weeks. Mice less than eight weeks old had a shorter incubation period but lower cyst production.

At the peak of infection, the cleaned and concentrated extraction of the feces of an eight-week old mouse would generally yield more than 2×10^6 cysts per day. This number could,

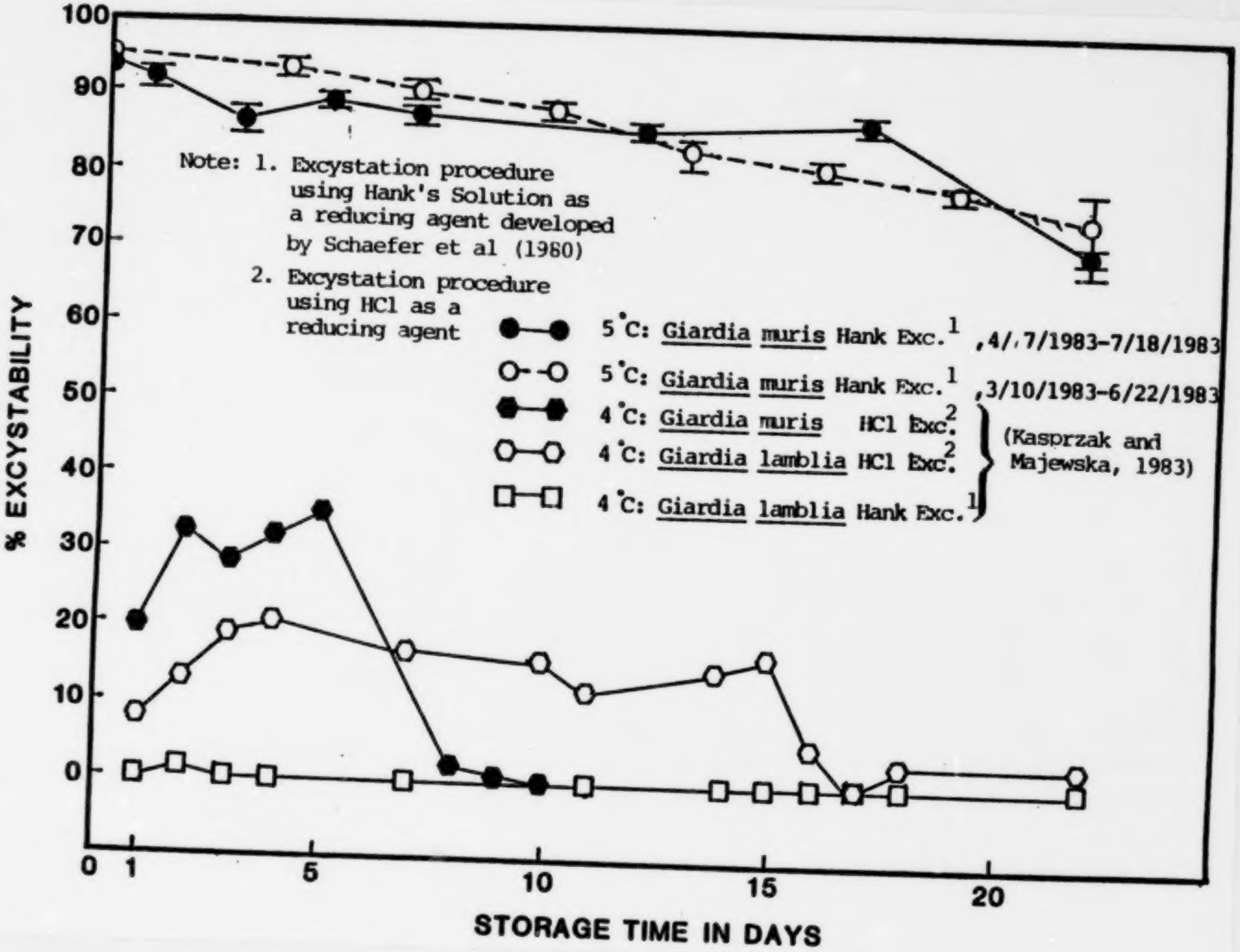


Figure 4. Comparison of excystation of _Giardia_ _muris_ and _Giardia_ _lamblia_.

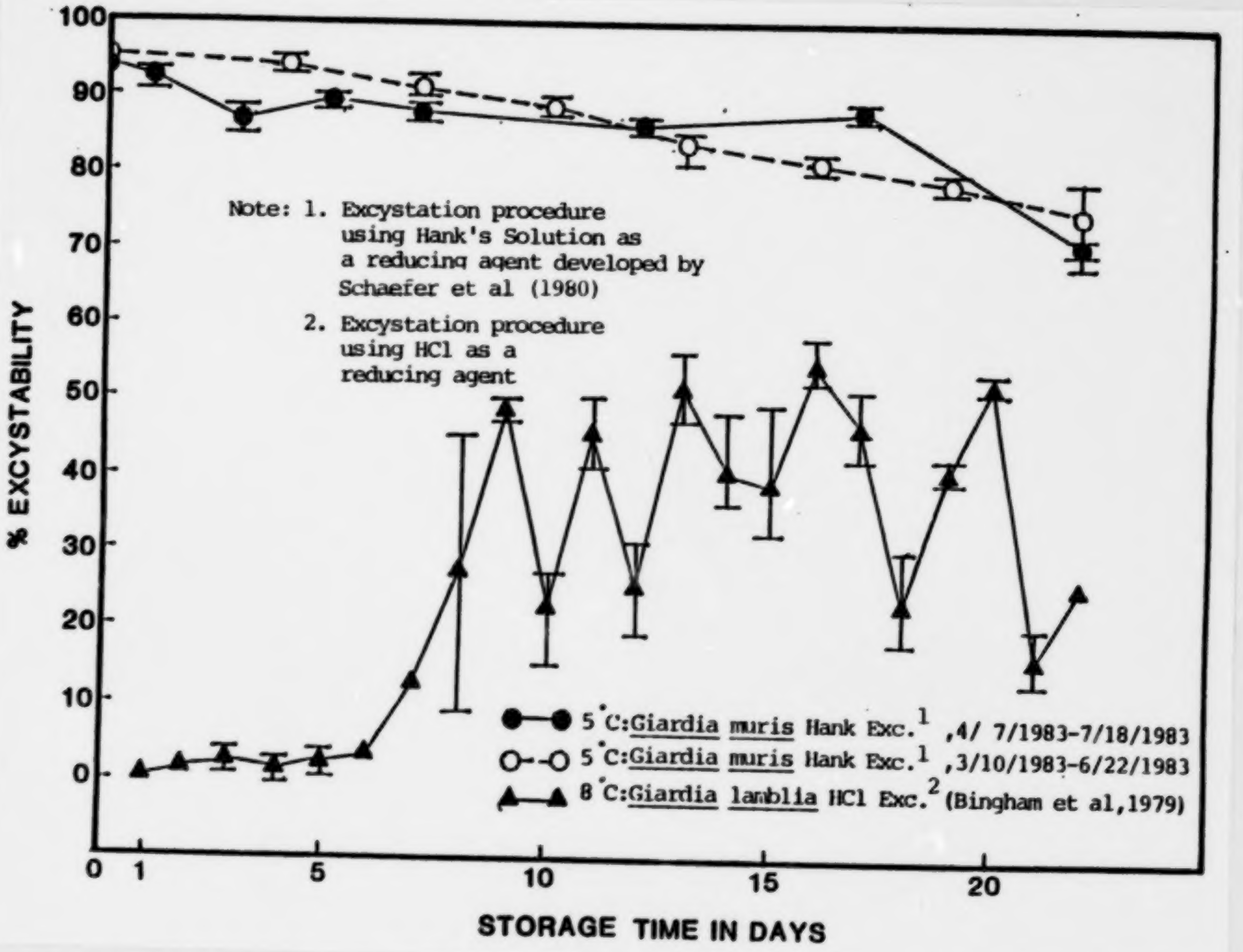


Figure 5. Fluctuation of excystation of *Giardia muris* and *Giardia lamblia*.

however, decrease significantly if a concurrent parasitic infection was present. (See Figure 6.) Cyst production also seemed to be adversely affected if the bedding material was not kept moist during the period of cyst collection.

Collection and Preparation of Giardia Cysts--

Fecal pellets were collected from the cages of infected mice. Cysts were then separated from the feces using a sucrose gradient technique (DeWalle et al., 1983) which was a modification of the method used by Sheffield and Bjorvatn (1977).

To extract the cysts, fecal material was emulsified in distilled water and passed through three or four layers of cheesecloth to remove the largest particles from the suspension. The filtrate containing the cysts and some debris was then centrifuged several times to separate out the very fine particles which otherwise would have become trapped with the cysts on top of the sucrose solution in the final separation step. Following the washing of the cysts to remove any trace of the sucrose solution, the cysts were stored at 5°C until needed. A detailed outline of the procedure can be found in Figure 7.

Excystation--

The excystation procedure used to test the viability of G. muris cysts was developed by Rice and Schaefer (1981). The test includes: an induction phase, an excystment step and microscopic determination of viability.

The excystation procedures for G. lamblia and G. muris were almost identical, the only difference being in the induction phase. Unlike the G. muris induction phase which did not require pH adjustment, the pH of the G. lamblia suspension was lowered to pH 2 for induction. The cysts were then incubated at 35°C for 30 minutes in a supplemented Hank's solution to which sodium bicarbonate ($NaHCO_3$) was added before placing the capped tube in the water bath. Following incubation, the cysts were concentrated by centrifugation.

The excystment phase consisted of exposing the cysts to the excystment medium and transferring them to a depression slide. The slide was sealed with a cover slip and incubated for 30 minutes in the inverted position in a 35°C incubator. After incubation, the percentage of excysted Giardia cysts was determined by examining the slide with an inverted microscope. Normally 8 to 16 fields were counted. Each excysted cyst produced two trophozoites that could be seen free-swimming in the solution or attached to the cover slip. The percent excystation was calculated by the following equation:

$$\%\ \text{Excysted} = \frac{(\text{Trophozoites}/2)}{(\text{Trophozoites}/2 + \text{Intact Cysts})}$$

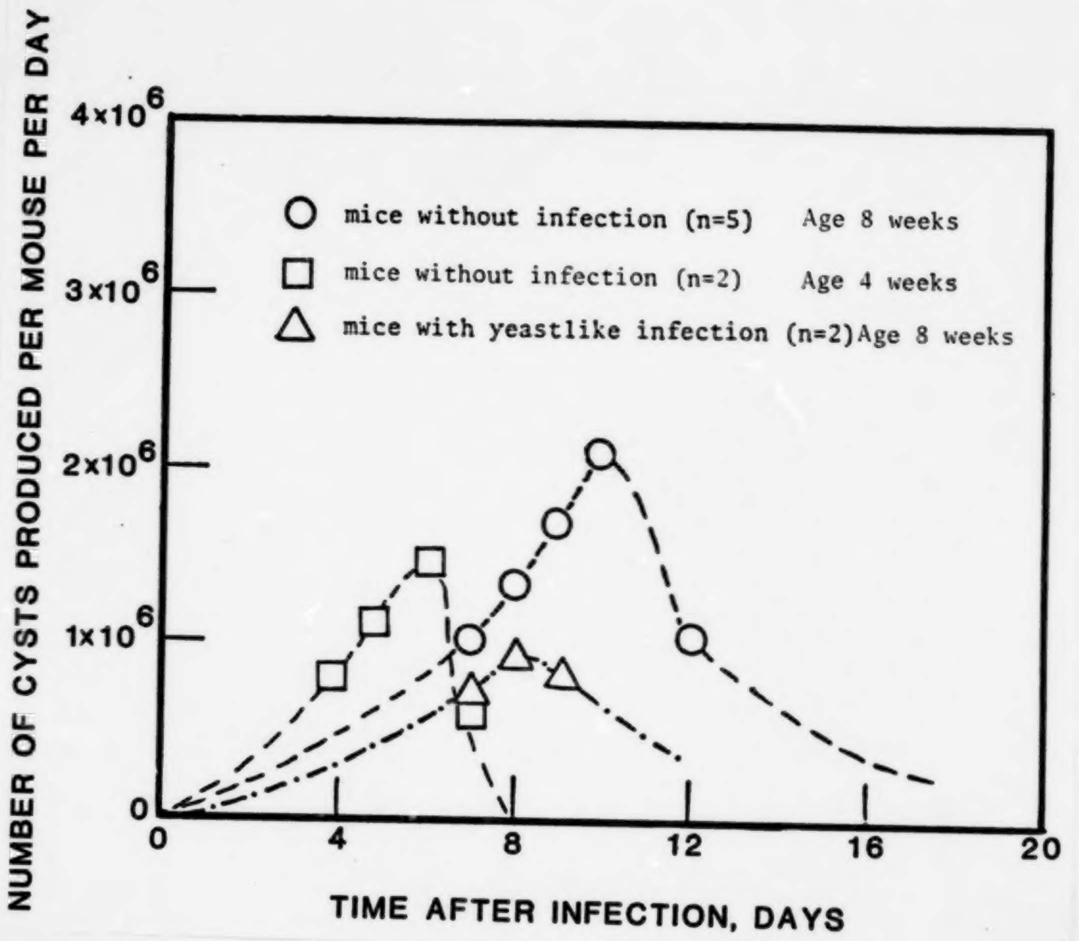


Figure 6. *Giardia muris* production in feces of infected mice.

Prepare and examine a fecal smear under the microscope to ascertain presence of cysts

↓

Transfer 12 g of feces to a 2000 mL beaker. Add distilled water to 500 mL and homogenize.

↓

Pour suspension through 3 or 4 layers of cheesecloth. Transfer liquid to 250 mL centrifuge bottles and centrifuge at 650 γ (2100 rpm) for 2 minutes.

↓

Aspirate off supernatant and resuspend sediments with distilled water. Pour into four 50 mL conical bottom tubes, make volume to 45 mL in each tube.

↓

Wash by centrifuging at 650 γ (2100 rpm)for 2 min. Discard supernatant and resuspend sediments. Repeat using 2 drops of Tween-80 liquid per 15 mL of suspension.

↓

Wash with distilled water until supernatant is reasonably clear.

↓

Resuspend sediments in 25 mL distilled water and layer onto 25 mL, 1.0 M sucrose, in two 50 mL tubes. Centrifuge at 800 γ (2400 rpm) for 10 minutes.

↓

Aspirate off 3/4 of supernatant appearing above the band of cysts. Pour remainder of suspension into a 50 mL tube and centrifuge at 800 γ (2400 rpm) for 2 minutes.

↓

Aspirate off supernatant and resuspend cysts at the bottom and repeat washing procedure twice.

↓

Resuspend final sediments and store at 5°C.

Figure 7. Extraction procedure for _Giardia_ cysts

A more detailed outline of the excystation procedure can be found in Figure 8.

Cyst Enumeration--

Only microscopic counting was used to determine cyst concentrations. This procedure allowed each cyst particle to be properly identified before being counted since, frequently, there were other particles present that resembled cysts both with respect to size and to shape. The counting chamber used was a hemacytometer, Clay Adams Model 4011 Spencer Bright-Line Counting Chamber. It had a volume of 0.9 mm^3 which meant that the number of cysts counted within the grid area had to be multiplied by 1111 to give the actual number per mL.

Normally 5 to 20 subsamples were counted from each cyst suspension. The lower number would be typical for suspensions to be used in experiments when viability was the primary interest. However, if the total number of cysts before and after the test were the main concern, a larger number of subsamples had to be examined for a more accurate determination of cyst density.

A total of approximately 5×10^4 cysts were used per excystation.

Zeta Potential--

A series of tests were conducted to determine the electrophoretic mobility (EM) and zeta potential (ZP) of Giardia cysts at different pH values and to establish the pH of the isoelectric point. The organisms used for these experiments originated from the same stool specimen. However, one half of the cyst suspension was fixed with formalin while the other was left unchanged. To investigate whether any changes the organisms might undergo with aging would affect their electric potential, the tests were repeated four different times at one month intervals. During this time the cysts were stored at 5°C.

The experiments were conducted with a Zeta-Meter (Zeta-Meter, Inc. New York, N.Y.) using a plexiglass Riddick type II UVA electrophoresis cell with a 4.4 mm diameter cell tube and cell constant of 62. The cell was equipped with a molybdenum anode and a platinum-iridium cathode spaced 10 cm apart. The cyst particles were tracked for a distance of 98 µm along a line 0.147 diameters from the tube wall where the electroendosmotic flow was zero. During the experiments conductance ranged from 39 to 4650 µmhos/cm and the corresponding applied voltage from 300 to 70 volts. The EM was calculated as:

$$EM = \frac{\text{Tracking Distance/Tracking Time}}{\text{Volts Applied/Electrode Spacing}} \ (\mu m/sec/volt/cm)$$

G. lamblia

↓

To a 15 mL centrifuge tube add 0.5 mL cyst suspension, 5mL HCl, 2.5 mL supplemented Hank's solution at 35°C and 2.5 mL 1.0M $NaHCO_3$ at 35°C. Cap tube.

G. muris

↓

To a 15 mL centrifuge tube add 0.5 mL cyst suspension, 5mL supplemented Hank's solution at 35°C, and 5mL 1.0M $NaHCO_3$ at 35°C. Cap tube.

↓

Vortex and incubate at 35°C for 30 minutes.

↓

Centrifuge at 350 γ for 2 minutes.

↓

Aspirate off supernatant and discard. Add 10 mL trypsin/Tyrode solution at 35°C, vortex to resuspend sediments.

↓

Centrifuge at 350 γ for 2 minutes.

↓

Aspirate off supernatant and discard.

↓

Add 0.5 mL trypsin/Tyrode solution at 35°C, vortex to resuspend sediments.

↓

Place suspension in depression slide, cover and seal with vaspar.

↓

Invert slide and incubate at 35°C for 30 minutes.

↓

Visually determine number of trophozoites, viable and non-viable cysts with inverted microcope.

Figure 8. Excystation procedure for *Giardia lamblia* and *Giardia muris* cysts.

The relationship between EM and ZP is described by the Helmholtz-Smulochowski formula:

$ZP = EM4\pi\nu/D$ (millivolts)

ν = viscosity of liquid in poises at the test temperature (units)
D = dielectric constant of liquid at the test temperature (units)

Assessment of *Yersinia enterocolitica* Viability

Source/Identification--

Three strains of fully virulent *Yersinia enterocolitica* were derived from human patients suffering from chronic gastrointestinal disease, manifested largely by recurrent and sporadic diarrhea or by acute episodes of ileitus. The three strains were labelled with respect to an antibiotic classification. Antibiotic classifications were as follows: *Y. enterocolitica* numbers 101, 102 and 103.

For UV batch analysis, all three strains were studied. For the inactivation runs at the Cedar River research field station, only *Y. enterocolitica* 103 was utilized. This organism is a member of the "American Classic" biogroup and contains plasmids at 41 and 73 MDal. The American Classic biogroup is composed of *Y. enterocolitica* that are indol positive or negative, usually delayed positive in citrate utilization and negative for mucate. Styrene is closely associated with virulence in "Classic" isolates of *Y. enterocolitica*. Rhamnose, raffinose and melibiose are not fermented at any temperature.

To study the effect of the presence of plasmids on UV inactivation, the plasmids from *Y. enterocolitica* 103 were "cured" (removed) from the virulent organism by rapid subculturing at 37°C and plating on magnesium oxalate agar for rapid growers - a subpopulation of *Y. enterocolitica* which are avirulent and plasmid-less.

Another population of avirulent, plasmid-less *Y. enterocolitica*, antibiotic strain 158, was studied. *Y. entercolitica* 158 falls within the biogroup Botzler-Bottone-Chester (B-B-C) which is considered a typical, environmental strain, nonpathogenic to humans. They have positive reactions for the sugars rhamnose, raffinose, and melibiose only at temperatures below 35°C. They are gelatin hydrolysis positive and styrene negative. For some B-B-C strains, less than 50% show delayed positive for lactose.

Seed Production--

Strains of *Y. enterocolitica* were grown in pure culture in Tryptical Soy Broth with Yeast Extract (TSB/YE) to logarithmic growth. At this time, several mL of culture were transferred to agar slants with sheets of solidified TSB agar, gently distributed across the media surface and incubated for 48 hours at 24°C. Cells were then harvested and diluted to about 30 mL with APHA Buffered Water (pH 7.0) supplemented with 0.1% surfactant (Tween 20). The diluted cells were centrifuged at 10,000 γ at 4°C for 20 minutes. The supernatant was discarded, either by pouring or aspiration. Cells were resuspended in 10 mL of new diluent, washed out into 30 mL volumes, and resuspended as described above. Three separate washings were performed.

After the final washing and sedimentation, the cells were diluted to about 10 mL with sterile distilled water, completely homogenized, and placed in a 4°C refrigerator. Cells were aged at least two weeks, preferably one month, before they were rewashed as illustrated previously. Concentration of the stock solution was determined by plating appropriate dilutions of *Y. enterocolitica* on Tryptical Soy Agar with Yeast Extract (TSA/YE). These plates were incubated for 60 hours at 24°C after which colonies were counted.

Enumeration--

Three separate media for isolating and enumerating *Y. enterocolitica* were studied because of the lack of an acceptable standardized method. The first two media, "MacTween" and mYE, required membrane filtration of the sample while the third media, Tergitol-7, was inoculated by surface spreading.

MacTween MPN--Appropriate dilutions of sample were passed through 0.45 μm filters and then aseptically transferred into tubes containing Selenite-F[1]. These tubes were then incubated at 4°C for 21 days for the purpose of cell enrichment. After incubation, the filters were placed on "MacTween" agar - a media composed of MacConkey agar plus $CaCl_2$ with Tween 20 added. The MPN was then calculated according to the number of positives for each dilution level. *Y. enterocolitica* colonies were identified as flat, wrinkled, and surrounded by precipitate zones of insoluble soaps.

mYE MPN--Serial dilutions of *Y. enterocolitica* samples were filtered through 0.45 μm membrane filters, transferred to sterile pads saturated with mYE recovery broth, and incubated at 25°C for 48 hours. Following incubation, the membrane was transferred to Lysine-Arginine agar and incubated anaerobically for one hour at 35°C. Afterwards, the membrane was transferred to a sterile pad saturated with Urease broth and incubated for 5-10 minutes at 25°C. Positive *Y. enterocolitica* were identified as green to blue colonies on Urease broth and which had been observed as yellow colonies on Lysine-Arginine agar.

---

[1] Selenite-F was prepared as specified in the Compendium of Methods for the Microbiological Examination of Foods, 1976.

Tergitol-7--Tergitol-7 was prepared by dissolving 33 g of Tergitol-7 (GIBCO) in one liter distilled water with the pH adjusted to 7.2. Non-used Kreb's urea cycle amino acids, such as lysine or arginine, were added to the media along with urea. One mL and 0.1 mL volumes of diluted *Y. enterocolitica* samples were surface spread over Tergitol-7 base plate agar and incubated at 24°C for 48 hours. Colonies of *Y. enterocolitica* cells were bright pink (urease positive) with an alkaline halo.

Assessment of Escherichia coli Viability

Source/Identification--

Two different strains of *E. coli* were evaluated in the batch UV studies: a Nalidixic Acid resistant (NAR) strain and a non-NAR strain.

E. coli-NAR were obtained from the effluent of the METRO (Municipality of Metropolitan Seattle) West Point Wastewater Treatment Plant by plating aliquots of effluent onto media which contained 25-30 mg/L Nalidixic Acid. *E. coli*-NAR was chosen as a laboratory model of an antibiotic-resistant coliform because its mode of action is not plasmid-associated. As the resistance is innate, it will be passed to its progeny and not be exchanged by donor-recipient conjugations.

Non-NAR *E. coli* strains were obtained from the ATCC and are registered under title number 25922. Non-NAR *E. coli* behaved like typical coliforms on indicator media.

Seed Production--

Seed production for *E. coli* strains was the same as described for the seed production of *Y. enterocolitica*.

Enumeration--

Three different media were used to isolate and enumerate the two different strains of *E. coli*. The first two media, Eosin Methylene Blue (EMB) and m-ENDO, are commonly used media for enumerating coliforms from water samples. Both media were inoculated with membrane filters. The third media, Tergitol-7 TTC, was utilized to differentiate between *E. coli*-NAR and background coliforms. Water samples were surface spread on Tergitol-7 TTC agar.

Eosin Methylene Blue (EMB)--Samples were filtered through 0.45 µm membrane filters and transferred to pads saturated with EMB broth. EMB broth was prepared as reported in Standard Methods (1975).

m-ENDO--Samples were filtered through 0.45 µm membrane filters and transferred to pads soaked with m-ENDO broth. m-ENDO was prepared as described in Standard Methods (1975). When it was desired to grow only E. coli-NAR, Nalidixic Acid was added at a concentration of 25 mg/L.

Tergitol-7 TTC--Tergitol-7 TTC medium was prepared as described for Y. enterocolitica with the supplemental addition of 30 mg/L triphenyltetrazolium chloride (TTC). For the identification of E. coli-NAR, 25 mg/L Nalidixic Acid was directly incorporated into the base of Tergitol-7 agar. One mL and one-tenth mL sample volumes were applied to the medium by surface spreading. E. coli colonies appeared yellow in color while all other organisms formed red colonies.

Assessment of Bacillus subtilis Viability

Source--

The pure culture of B. subtilis (ATCC 6633) used in this study was obtained from the American Type Culture Collection, Maryland (ATCC). The strain culture was maintained as colonies on nutrient agar. A medium containing nutrient broth (Difco), 16 g/L; $MgSO_4 \cdot 7H_2O$, 0.5 g/L; KCL, 2.0 g/L; 10^{-3}M $Ca(NO_3)_2$, 10^{-4}M $MnCl_2$, 10^{-6} M $FeSO_4$, and 0.1 % glucose was used for cell growth. The medium is designated as "2 x SG" (Leighton and Doi, 1971).

For sporulation experiments, one colony was inoculated into 20 mL of 2 x SG and incubated with shaking for 24 hours at 37°C. The cells were harvested at the desired periods of growth and centrifuged in distilled water twice at 5000 γ for 10 minutes. The cell suspension was then placed in 65°C water bath for an hour in order to kill the remaining vegetative cells without affecting spore viability. The spore culture was then stored at 5°C until use.

Enumeration of B. subtilis Spores--

The bacteria spread plate count method (Standard Methods, 1980) was employed for enumeration of B. subtilis spores using nutrient agar as a growth medium. After the three dilutions of the test samples were made, 0.1 mL of each sample was spread on three agar petri discs to yield triplicate results. The discs were then incubated at 37°C for 24 hours. The number of colonies of the spores was identified and counted according to the Abbott Laboratories' manual (1982), and used for back-calculation to determine the original spore concentration in each water sample.

EQUIPMENT

UV Contactors

UV Batch Reactor--

The UV batch unit was a pyrex glass vessel 10.2 cm (4 in.) in diameter and 16 cm (6.3 in.) deep (Figure 9). It was fitted with a plexiglass cover to which the quartz lamp holder and two sampling needles were attached. A total of four stainless steel

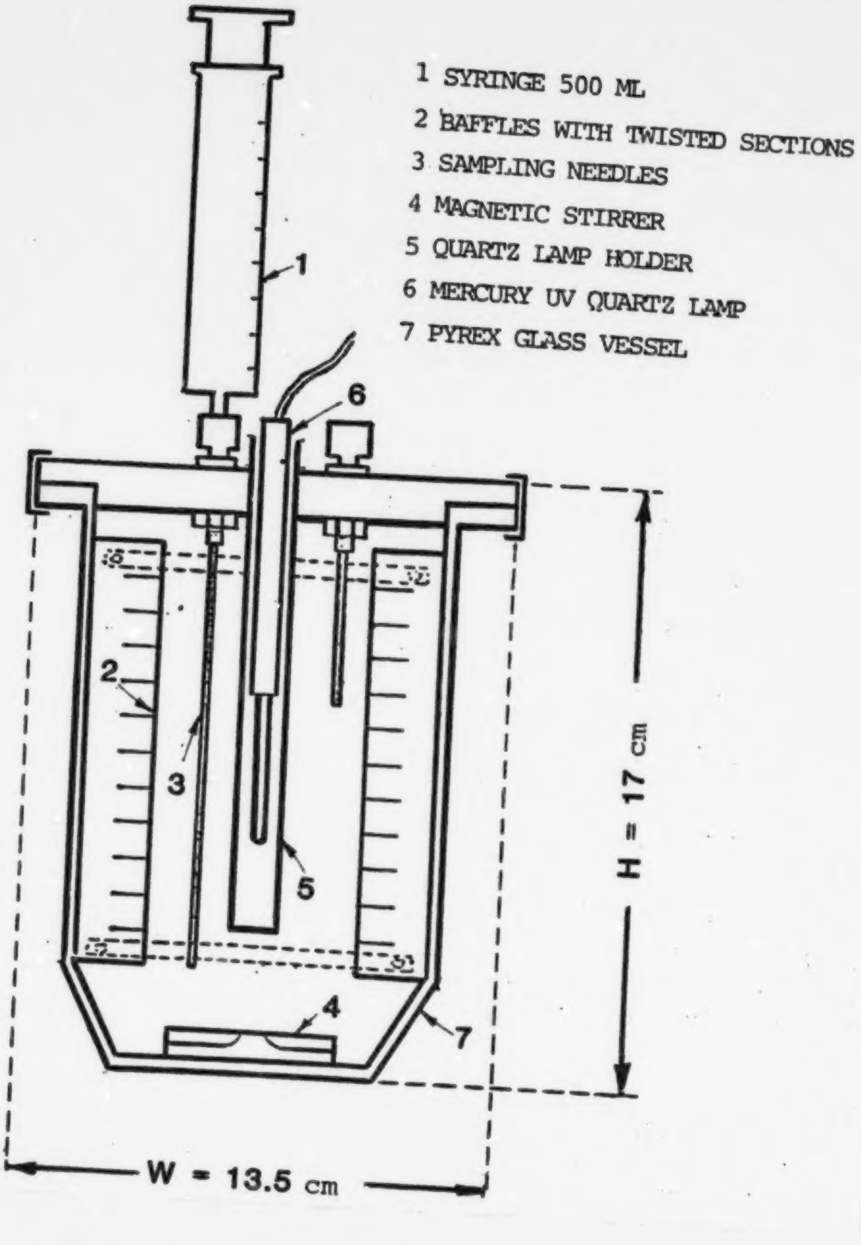

1 SYRINGE 500 ML
2 BAFFLES WITH TWISTED SECTIONS
3 SAMPLING NEEDLES
4 MAGNETIC STIRRER
5 QUARTZ LAMP HOLDER
6 MERCURY UV QUARTZ LAMP
7 PYREX GLASS VESSEL
1
6
2
3
5
4
7
H = 17 cm
W = 13.5 cm

Figure 9. Laboratory Batch Reactor (Pen Ray) used in the UV inactivation experiments.

baffles or deflectors were placed at the inside wall of the container. Along the edge of the deflectors, small incisions were made and every other section created by the incisions was twisted. The purpose was to induce mixing, thereby preventing laminar flow in the vertical direction and possible laminar sheet flow around the light source.

To maintain a uniform distribution of organisms in the reactor, the suspension was stirred continuously by a turbine stirrer in conjunction with a "Corning, PC-353" magnetic stirrer. The magnetic mixer was operated at 60 percent of maximum speed.

The organisms were irradiated with a Pen Ray 115 volt, 5.5 watt UV light source. Before each test, the Pen Ray was "warmed up" for five minutes in order to reach an equilibrium temperature and then inserted into the reactor's quartz lamp holder. During this time, the suspension of organisms in the reactor was continually mixed to obtain and maintain an homogeneous mixture.

Continuous Flow Reactor, Pure Water Systems (PWS), Model I-30--
One of the commercial UV disinfection units selected for the study was a Model I-30 from Pure Water Systems, Inc., Fairfield, New Jersey. This stainless steel unit was 15.2 cm (6 in.) in diameter and 58.4 cm (23 in.) long. It was mounted horizontally on a 4.5 cm (1.75 in.) tall base as shown in Figure 10a. The experimental setup utilizing the Pure Water Systems UV unit is shown in Figure 10b.

Water entered the disinfection chamber near the bottom at one end, flowed through the reactor in a helical flow pattern and exited at the opposite end. The effective volume of the chamber was 2.4 L. To prevent air from accumulating inside, a bleed valve was installed in the top of the unit.

Six 115 volt, 40 watt UV lamps, capable of producing 30 mW/cm^2 of energy, were separated from the water with quartz jackets. A sensor on top of the unit monitored lamp output and alerted the operator to the need for UV lamp replacement. In addition, pilot lights on the power supply box provided a quick check on lamp operation.

The quartz jackets were cleaned by a compressed air powered wiper, moving from the outlet side of the unit to the inlet side and back. An adjustable timer in the power supply box regulated the stroke frequency.

Continuous Flow Reactor, Ultraviolet Technology (UTI), Model 40L--
The second commercial disinfection unit used in this investigation was a Model 40L from Ultraviolet Technology, Inc., San Marcos, California. The overall dimensions were 116.8 cm (46 in.) long, 23.5 cm (9.25 in.) wide and 20.3 cm (8 in.) high. The outer enclosure was made of anodized aluminum. Inside was a 3.8

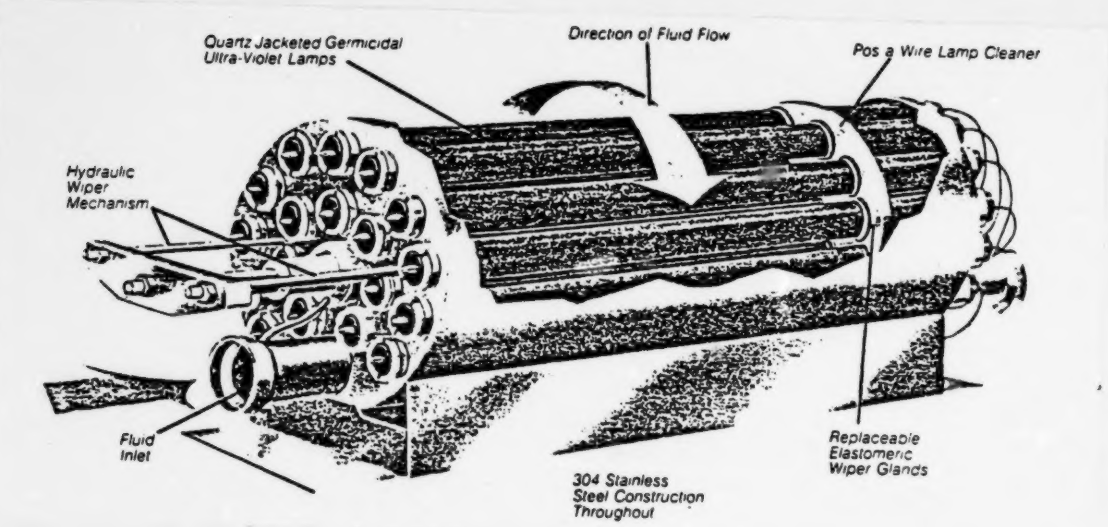

Quartz Jacketed Germicidal Ultra-Violet Lamps
Direction of Fluid Flow
Pos a Wire Lamp Cleaner
Hydraulic Wiper Mechanism
Fluid Inlet
304 Stainless Steel Construction Throughout
Replaceable Elastomeric Wiper Glands

Figure 10a. Pure Water Systems continuous flow reactor, Model I-30

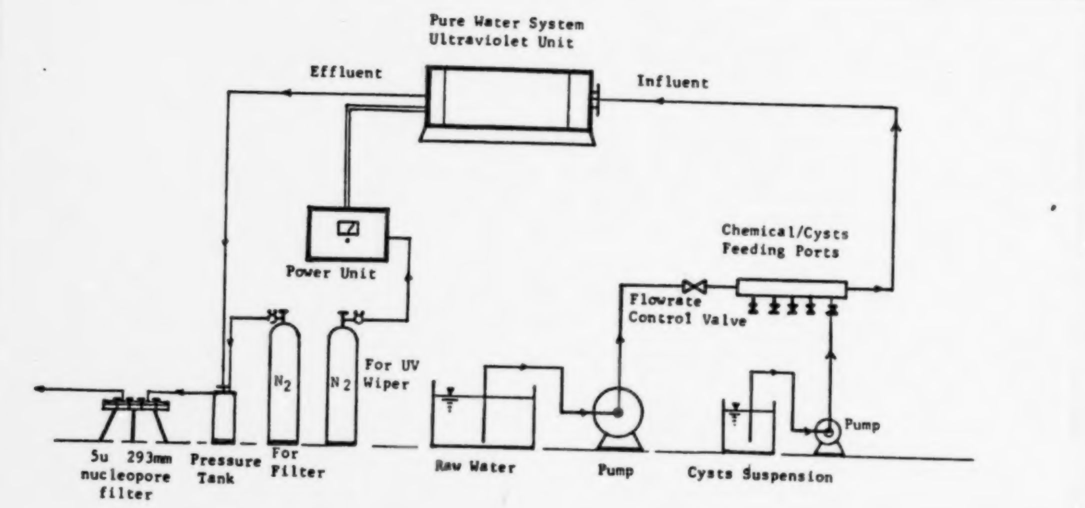

Pure Water System Ultraviolet Unit
Effluent
Influent
Power Unit
Chemical/Cysts Feeding Ports
Flowrate Control Valve
N2
N2
For UV Wiper
5u 293mm nucleopore filter
Pressure Tank
For Filter
Raw Water
Pump
Cysts Suspension
Pump

Figure 10b. The experimental setup for UV disinfection study.

cm (1.5 in.) ID fluid carrying teflon tube, surrounded by four UV lamps rated 50 watts at 120 volts. The inside of the enclosure served as a reflector of UV light. The distance from each lamp to the tube wall was 3.4 cm (1.3 in.) and the length of the teflon tube exposed to the germicidal lamps was 90 cm (35 in.) which corresponded to a volume of 1 L for the disinfection chamber. See Figures 11a and 11b.

Normal operation of the two commercial contactors included a 15 minute warm-up period for the mercury vapor lamps during which time the flow rate through the unit was adjusted to give the desired detention time. A series of conductivity tests were used to establish a relationship between flow rate and detention time and determine sampling times.

At various flow rates, a salt solution was injected at the in-line manifold which was later to be used for adding microorganisms, turbidity and color. Immediately following the manifold was a static mixer, Kenics Model 1/2-10-321-5 (Kenics Corporation, North Andover, Maine), to insure uniform distribution of all constitutents in the water as it reached the disinfection chamber. Throughout the salt addition, the conductivity of the UV unit effluent was monitored to determine the time required to reach a constant level. This information was used to select suitable sampling times during the ensuing disinfection experiments.

To arrive at the actual detention time of the contactor these tests were repeated, only this time, the conductivity of the influent to the reactor also was measured. By comparing the slope of the influent and effluent conductivity curves, the detention time through the reactor could be calculated.

To ascertain that factors other than the exposure to UV radiation did not contribute to the inactivation of the organisms, all experiments included a run with all UV lamps turned off (UV dosage = 0). This procedure established a base line to which the other runs would be compared.

After passage through the reactor 10 L samples containing the organisms were collected, concentrated and tested for viability as described earlier.

Excimer Laser--

The laser used to provide a coherent light source at several wavelengths was a model 100XR excimer multigas laser manufactured by Tachisto, Inc., Needham, Maine. The wavelengths chosen for UV evaluations were 193, 248 and 351 nm. Primarily work was done with 248 nm as a comparable wavelength to that emitted by mercury vapor lamps. The change in UV wavelength was generated by changing the gas mixture for the lasing medium. Thus, an argon/

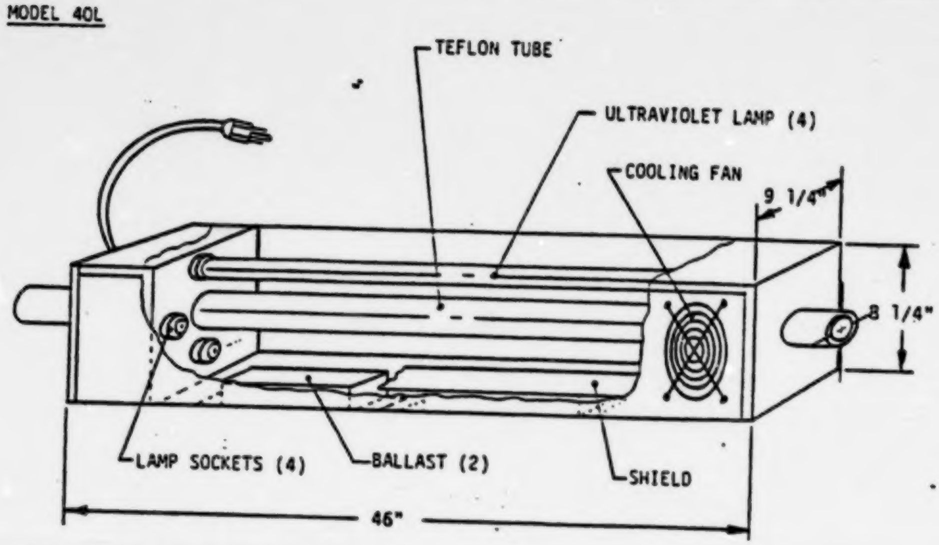

MODEL 40L
TEFLON TUBE
ULTRAVIOLET LAMP (4)
COOLING FAN
9 1/4"
8 1/4"
LAMP SOCKETS (4)
BALLAST (2)
SHIELD
46"

Figure 11a. Ultraviolet Technology continuous flow reactor, Model 40L.

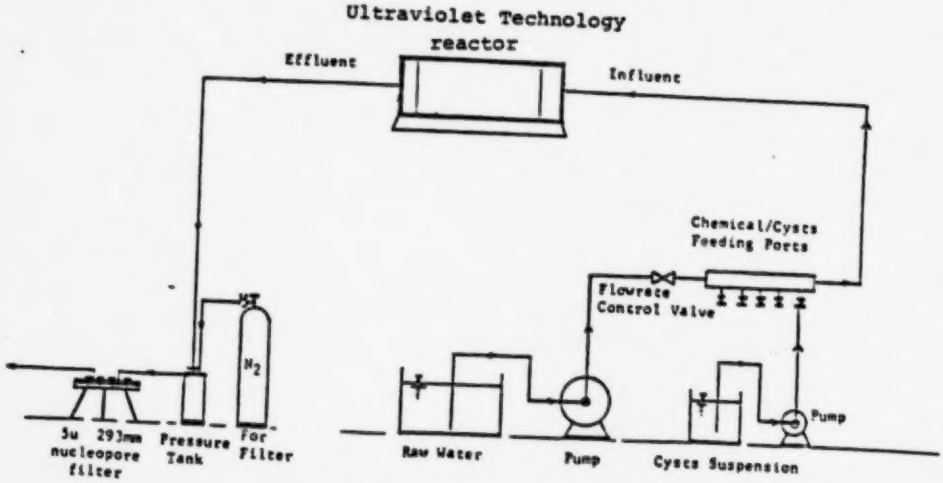

Ultraviolet Technology reactor
Effluent
Influent
Chemical/Cysts Feeding Ports
Flowrate Control Valve
N_2
5u 293mm nucleopore filter
Pressure Tank
For Filter
Raw Water
Pump
Cysts Suspension
Pump

Figure 11b. The experimental setup for UV disinfection study.

fluorine mixture was used for 193 nm, a krypton/fluorine mixture for 248 nm and a xenon/fluorine mixture for 351 nm.

The percentages of fluorine and inert gas used to provide the various wavelengths are indicated below. Helium was used as the major carrier to bring the mixture up to 100 percent pressure. The percentages are in terms of partial pressure.

ArF:	10% argon, 0.26% fluorine
KrF:	5% krypton, 0.26% fluorine
XeF:	0.63% xenon, 0.26% fluorine

The optimum pressure range for operation was given as 1900 to 2100 torr. The experiments reported herein were done at 2100 torr.

The laser generating unit is relatively large for laboratory equipment with a length of 137.8 cm (54.25 in.), width of 20.8 cm (28.20 in.), height of 34.3 cm (13.50 in.) and weight of 460 lbs. In addition, floor space is required for the gas bottles and valving supplying the lasing medium as shown in Figure 12b. In the laboratory arrangement used for these studies, the laser equipment was housed in a special roof laboratory and the fluorine bottle placed in a cabinet external to the laboratory with the gas supply piped back into the laboratory area so any potential leakage of fluorine gas would be external to the roof laboratory and to the general laboratory complex. The manufacturer's data states that the average power at the respective wave lengths are 550 mW at 193 nm, 838 mW at 248 nm, and 313 mW at 351 nm.

The concept of the excimer laser is that an electrical discharge emitted into an appropriate gas mixture such as krypton fluoride can produce a population of excited state molecules or excimers of which KrF* would be an example. These excimers subsequently decay with the emission of the UV radiation.

As shown in Figures 12a and 12b, the equipment for the laser is largely a matter of providing for proper gas mixtures and then providing controlled high voltage discharge to excite the gases. The pulsed UV beam exits from the unit through the output port (Figure 12b). The water to be disinfected by the UV must then intercept and absorb this beam.

Hydraulics of Reactors

The ability of disinfecting agents to meet established effluent standards is a function of several factors including (1) the agent's inherent ability to inactivate (biocidal characteristics), (2) mixing (mass transfer) of the disinfectant into the process stream and (3) the hydraulic dispersion characteristics of the reactor. Of these three factors, the last two are more amenable to control in design considerations. Depending on

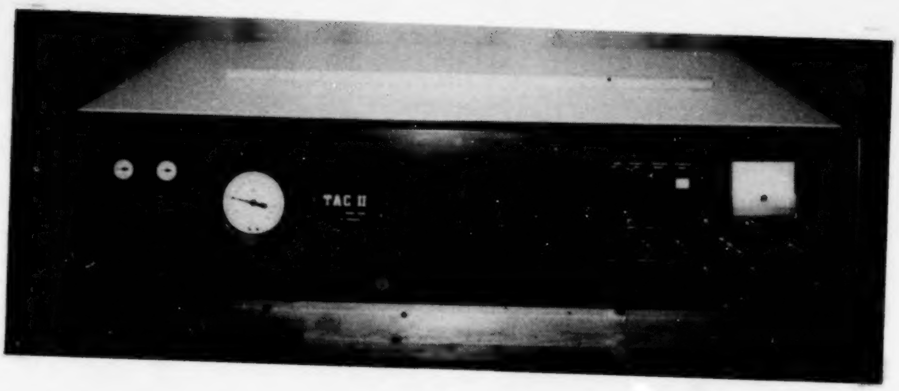


Figure 12a. Excimer laser, Tachisto Model 100XR.

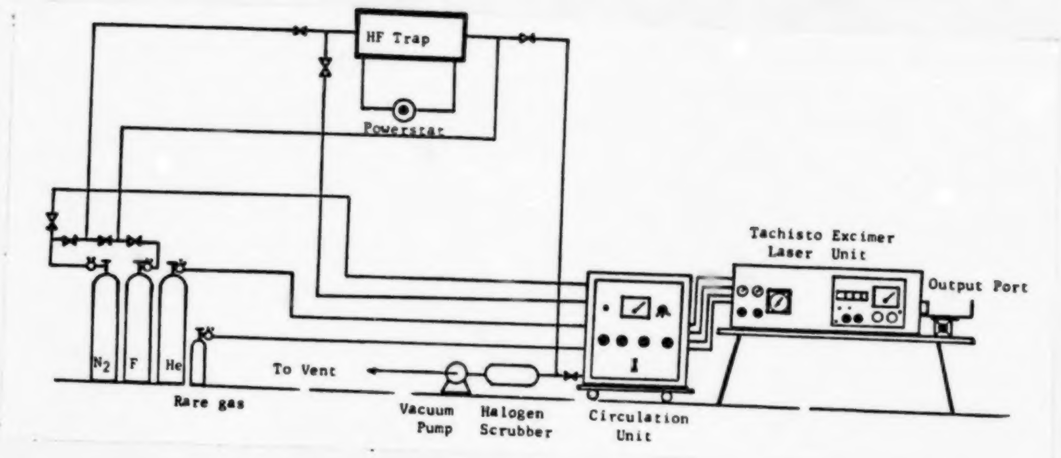

HF Trap
Powerstat
Tachisto Excimer Laser Unit
Output Port
N_2
F
He
Rare gas
To Vent
Vacuum Pump
Halogen Scrubber
Circulation Unit

Figure 12b. The experimental setup for laser-UV disinfection study.

the degree of disinfection, these factors take on different levels of importance for different disinfectants.

A California study of chlorination of wastewater effluents reported better disinfection at lower chlorine doses with optimization of initial mixing and use of plug flow contact tanks. Of eight full-scale chlorination contactors studied, the two worst performing contactors had flow patterns approaching complete mix, while the better contactors exhibited plug flow characteristics (Sepp and Bao, 1981). Mixing and hydraulic dispersion are also important considerations in ozone disinfection. Venosa and Opatken (1979) stated the necessity of maximizing ozone contactor efficiency because of the difficulty of maintaining high partial pressures of ozone above the process stream. Based on theoretical considerations, better ozone transfer efficiency is possible in a countercurrent plug flow reactor.

Unlike chlorine and ozone, ultraviolet radiation does not have problems with mixing. Its physical nature renders UV free of the problems of mass transfer. However, like chlorine and ozone, contactor flow dispersion has a significant impact on the ultimate ability of UV reactors to attain significant disinfection levels. This is especially true in light of UV's inability to produce a residual.

Several ultraviolet disinfection studies have shown that, as reactors approach plug flow, greater degrees of disinfection can be attained at lower doses, and therefore, lower costs. In a study by Severin et al. (1983, 1984), a completely mixed reactor could attain only a three-log reduction of E. coli at an average dose of 6820 mW-sec/cm^2, although log reductions greater than five were achieved in batch studies in a completely stirred treatment reactor (CSTR) at a much lower dose of 22.5 mW-sec/cm^2. Initial resistance of the microorganisms, rather than kinetic inactivation rates, appeared to be the limiting factor in establishing the disinfection limits of their completely mixed UV reactor.

Another study (Petrasek et al., 1980) reported that when the ratio of actual detention time to theoretical detention time (Ta/Tth) of a reactor dropped well below 1.0, disinfection was significantly hindered. Using a Kelly-Purdy UV reactor (fluorescent UV lamps suspended longitudinally above a sheet flow), flow depth in the reactor was varied to study the effect of Ta/Tth on coliform disinfection. For a dosage of 32 mW-sec/cm^2 applied to a 2.54 cm depth of flow with a Ta/Tth = 1.0, total coliform and fecal coliform concentrations were reduced 3.38 and 3.40 logs, respectively. In comparison, for a Ta/Tth = 0.77, (a dosage of 38 mW-sec/cm^2 applied to a 6.35 cm depth of flow) total coliform and fecal coliform reductions were only 2.29 and 2.25 logs, respectively. The difference, primarily a function of short-circuiting, appears to be expressed by the Ta/Tth ratio.

Johnson et al. (1982) reported that, when B. subtilus spores were used as a tracer for a thinfilm PWS-UV reactor, most surviving spores emerged from the UV contactor well before the average retention time. This unit exhibited both short-circuiting and tailing in residence time distribution (RTD) curves. Ta/Tth ratios were 0.75 and 0.79 for flow rates of 2.0 L/sec (32.3 gpm) and 0.6 L/sec (8.84 gpm), respectively. Because of short-circuiting, coliform reductions were limited to approximately 3 logs. In comparison, a second UV unit, which attained greater than 4.5 log reductions in coliforms, had significantly less short-circuiting and tailing. Ta/Tth ratios were 0.95 and 0.97 at flow rates of 2.9 L/sec (45.5 gpm) and 0.6 L/sec (8.84 gpm), respectively.

These studies clearly demonstrate the need to maintain nearly plug flow conditions in the disinfection reactor in order to attain effective disinfection. The total coliform maximum contaminant level (MCL) for potable water is so stringent that even small degees of short-circuiting through the reactor make it impossible to attain the necessary level of disinfection. Compensation for short-circuiting problems can be achieved by increasing the UV energy reaching the microorganisms, although this increase will result in increased operating costs, and make the reactor less attractive economically than alternative disinfectants.

Procedure--

A series of residence time distribution (RTD) curves were developed at various flow rates for both the plug flow reactors used in this study. A concentrated solution of NaCl was used as the tracer. A salt "spike" was injected into the UV treatment system through the manifold used to inject microorganisms, color and turbidity. Salt concentration, as measured by a conductivity meter attached to a single pole chart recorder, was determined just before the UV reactor (i.e., at the effluent of the inlet tubing) and directly after the reactor. This resulted in RTD curves for: 1) the inlet tubing (before reactor), and 2) the inlet tubing and UV reactor (after reactor). All RTD curves were evaluated in terms of the mean detention time (T), initial appearance of tracer (t_o), and the variance (S^2). The additive property of the variance and mean for flow through independent vessels was used to extract the characteristics of the RTD for the UV reactors:

$$T\ (\text{inlet + UV reactor}) = T\ (\text{inlet}) + T\ (\text{UV reactor})$$

$$S^2\ (\text{inlet + UV reactor}) = S^2\ (\text{inlet}) + S^2\ (\text{UV reactor})$$

Results--

Often RTD curves are normalized with respect to time and mass of the spike, such that the area under the curve is 1.0.

Figures 13a and 13b present normalized RTD curves for both UV reactors, using θ, the ratio of actual time to mean residence time. Visual comparison of the curves shows the dramatic dispersion which took place in the PWS-UV reactor. Dispersion in the PWS-UV is a result of both short-circuiting and tailing. Characteristics of the reactor vessel RTD curves are presented in Tables 1 and 2.

TABLE 1. HYDRAULIC DISPERSION CHARACTERISTICS OF PURE WATER SYSTEMS-UV REACTOR

Flow Rate (mL/sec)	Mean Detention Time (sec)	Standard Deviation (sec)	Dispersion Coefficient ($\Delta/\Gamma U$)
80.5	21.3	12.8	0.23
165	17.3	11.5	0.31
223	13.0	9.5	0.29
271	11.9	5.5	0.205
309	11.6	5.4	0.110
342	9.8	4.9	0.125

TABLE 2. HYDRAULIC DISPERSION CHARACTERISTICS OF ULTRAVIOLET TECHNOLOGY-UV REACTOR

Flow Rate (mL/sec)	Mean Detention Time (sec)	Standard Deviation (sec)	Dispersion Coefficient ($\Delta/\Gamma U$)
103	14.6	6.3	0.10
169	7.7	3.0	0.085
221	7.2	1.9	0.035
275	5.6	1.3	0.030
319	4.7	1.0	0.023
351	4.2	0.8	0.020

Dispersion also can be evaluated for individual reactors at different flow rates. As shown in Figure 14, dispersion was more pronounced at lower flow rates for both the PWS-UV and UTI-UV.

In Figure 14, reactor standard deviation, determined from RTD curves, is shown as a function of flow rate. For the PWS-UV reactor, the standard deviation decreased dramatically from 12.8 seconds at a flow rate of 100 mL/sec, to 5.6 seconds at 300 mL/sec. This substantial decrease appeared to be a product of both reactor hydraulics and the transition from laminar to turbulent flow, although the latter consideration was not confirmed.

Dispersion created by the UTI-UV, as measured by reactor standard deviation, was not significant at flow rates greater than 225 mL/sec. In fact, for the UTI reactor, standard deviation appeared to approach zero as flow rates increased, which was an indication of true plug flow at low detention times. Only at flow rates below 225 mL/sec did dispersion in the UTI-UV reactor contribute significantly to the calculated standard deviation.

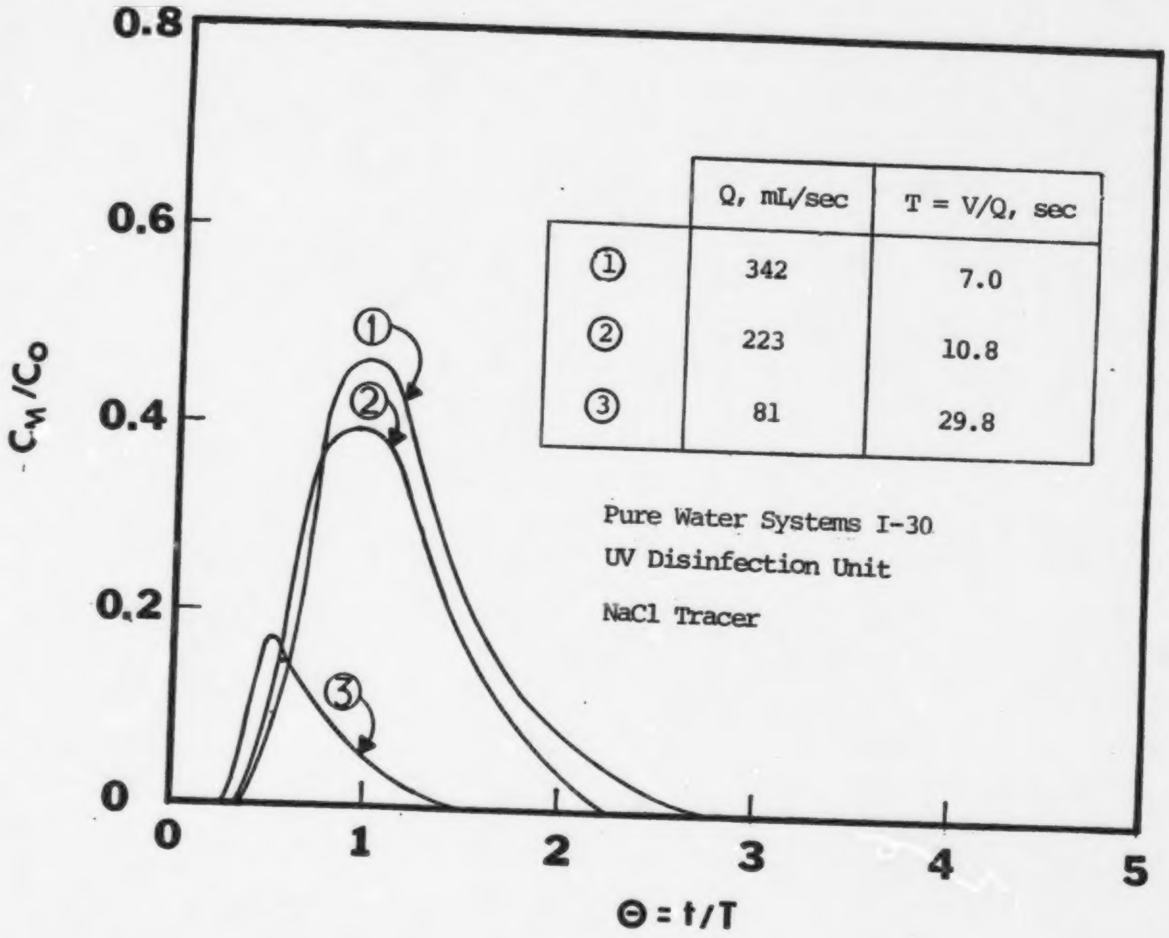


Figure 13a. Residence time distribution curves for Pure Water Systems UV unit.

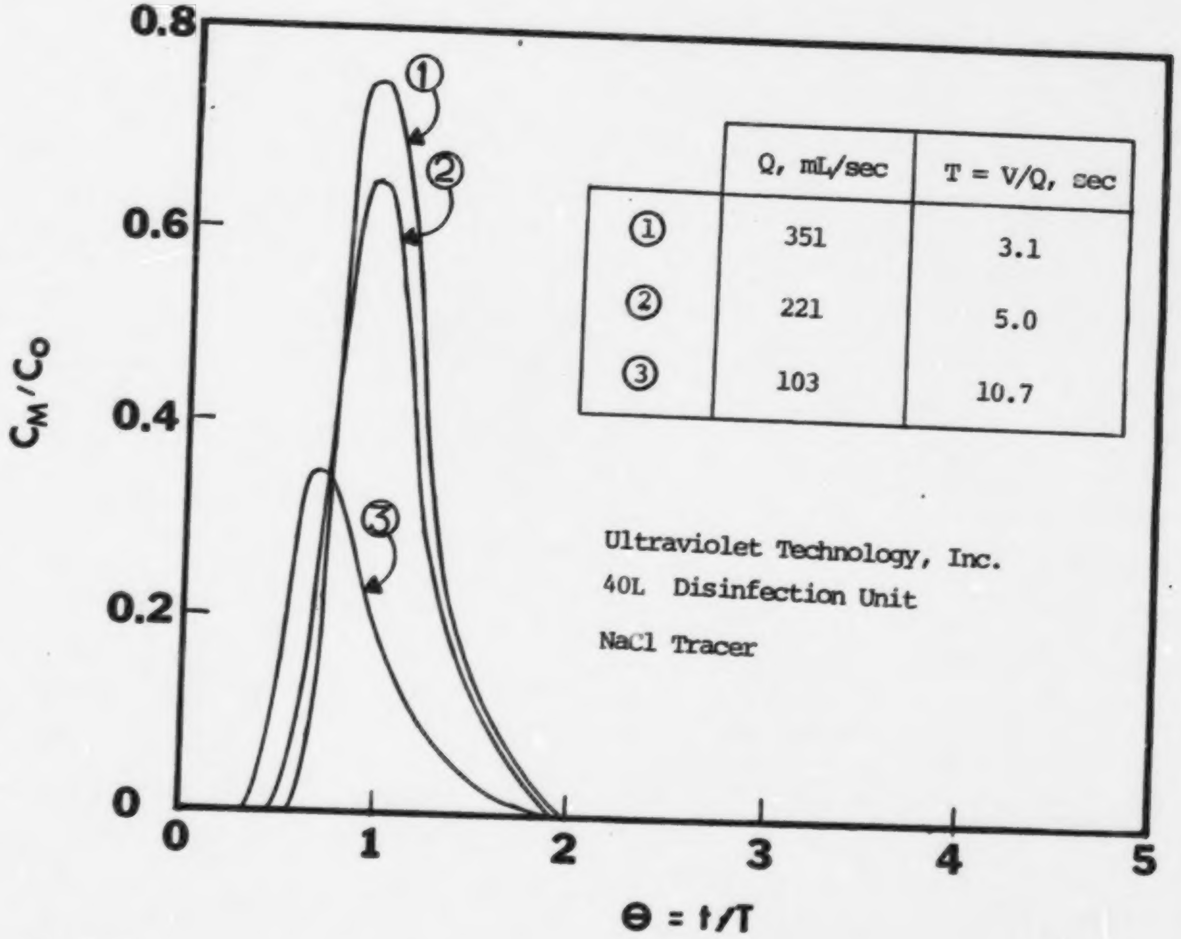


Figure 13b. Residence time distribution curves for Ultraviolet Technology UV unit.

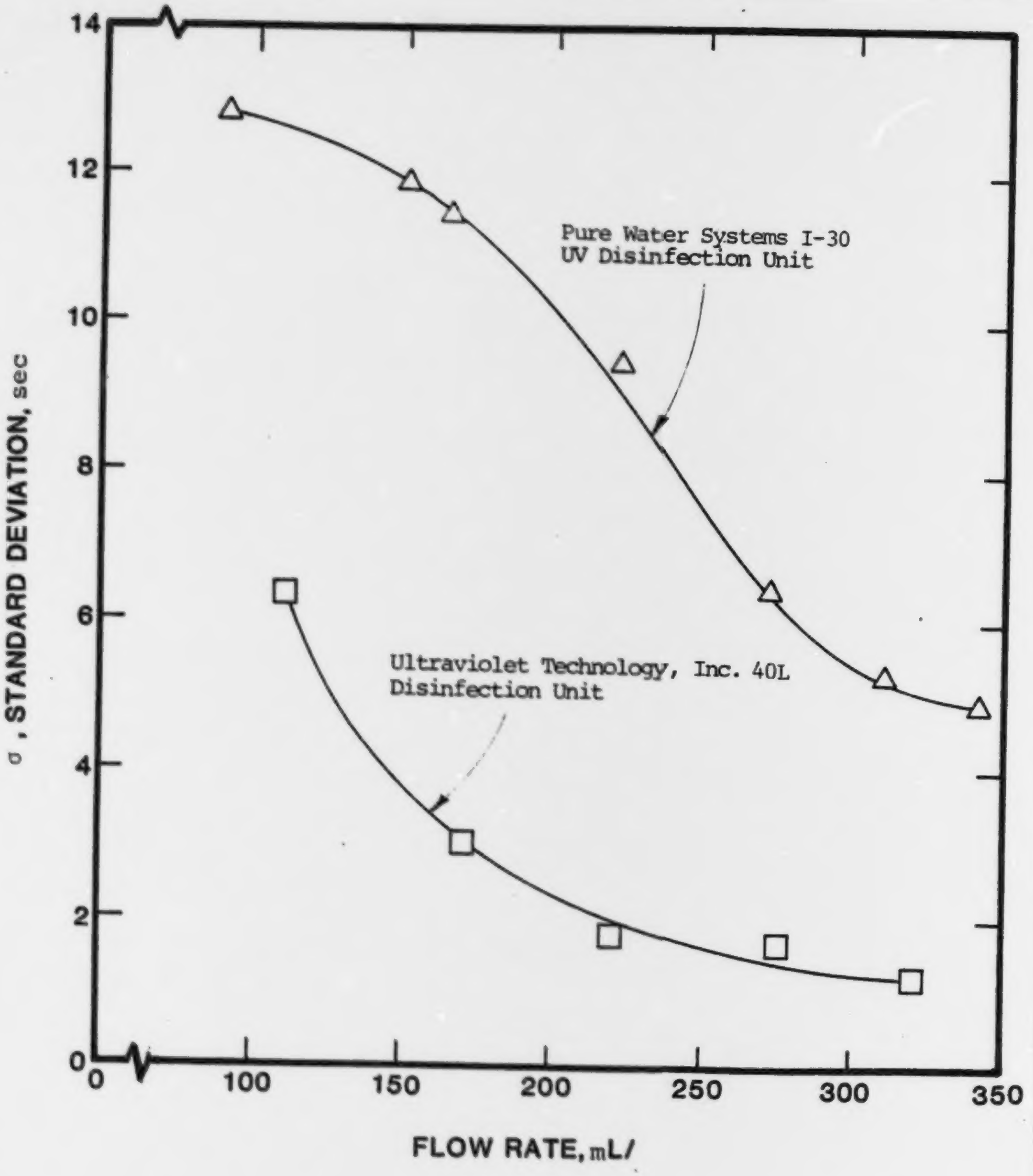


Figure 14. Flow characteristics of UV reactors as measured by standard deviation of residence time distribution (RTD) curves.

A more appropriate comparison can be made by relating reactor vessel standard deviation to theoretical detention time. Figure 15 depicts how the dispersion of the two reactors can affect disinfection levels. For all detention times studied, the UTI had a smaller reactor standard deviation than the PWS-UV unit. This relationship suggested that the UTI unit should attain greater levels of microorganism reduction than the PWS for the same energy expenditure (UV dose).

Another index often used to describe reactor hydraulics is the dispersion coefficient "δ". As δ approaches 0.0, dispersion is negligible and plug flow is attained. The further δ extends from 0.0, the greater the dispersion in the vessel. Dispersion coefficients, calculated for each RTD curve of the UV reactors, are presented in Tables 1 and 2.

Dispersion coefficients of the UTI unit were quite small, although they were not small enough to be approximated by a Gaussian or normal distribution as suggested by Levenspiel (1972). Below values of 221 mL/sec, the UTI dispersion coefficient slowly increases with decreasing flow rate. This same relationship is seen between flow rate and reactor standard deviation.

Pilot Plant

The water treatment pilot plant containing a direct filtration unit was housed in a 3.0x9.1 m (10x30 ft) trailer. The plant consisted of two separate but identical treatment trains, each designed for 3.8 L/min (1 gpm). Water was supplied to the plant by a 1/2 HP centrifugal pump. Two solenoid valves which could be operated completely independently of each other, controlled the flow to the two streams, thereby allowing for different treatment schemes to be evaluated side by side. Following each solenoid valve was a flow sensor, a five port manifold for adding turbidity, color, microorganisms, and chemicals for pH control, a needle valve for flow control, another five port manifold for coagulant addition, and an in-line mixer (Kenics Model 1/2-10-321-5, Kenics, Danver, MA). After passing the static mixers, coagulated water flowed directly into the first of the three flocculation compartments (see Figure 16).

Flocculators--

The flocculation unit was constructed from 1.9 cm (3/4 in.) plywood sheeting and coated with fiberglas. Each treatment stream contained three compartments, 30.5x30.5 cm (12x12 in.) wide and 61 cm (24 in.) deep. Water entered the flocculation compartment at the bottom and was discharged through a standpipe connected to an elbow at the bottom on the opposite end. The water depth in the compartments, and hence the detention time, could be controlled by varying the length of the standpipes.

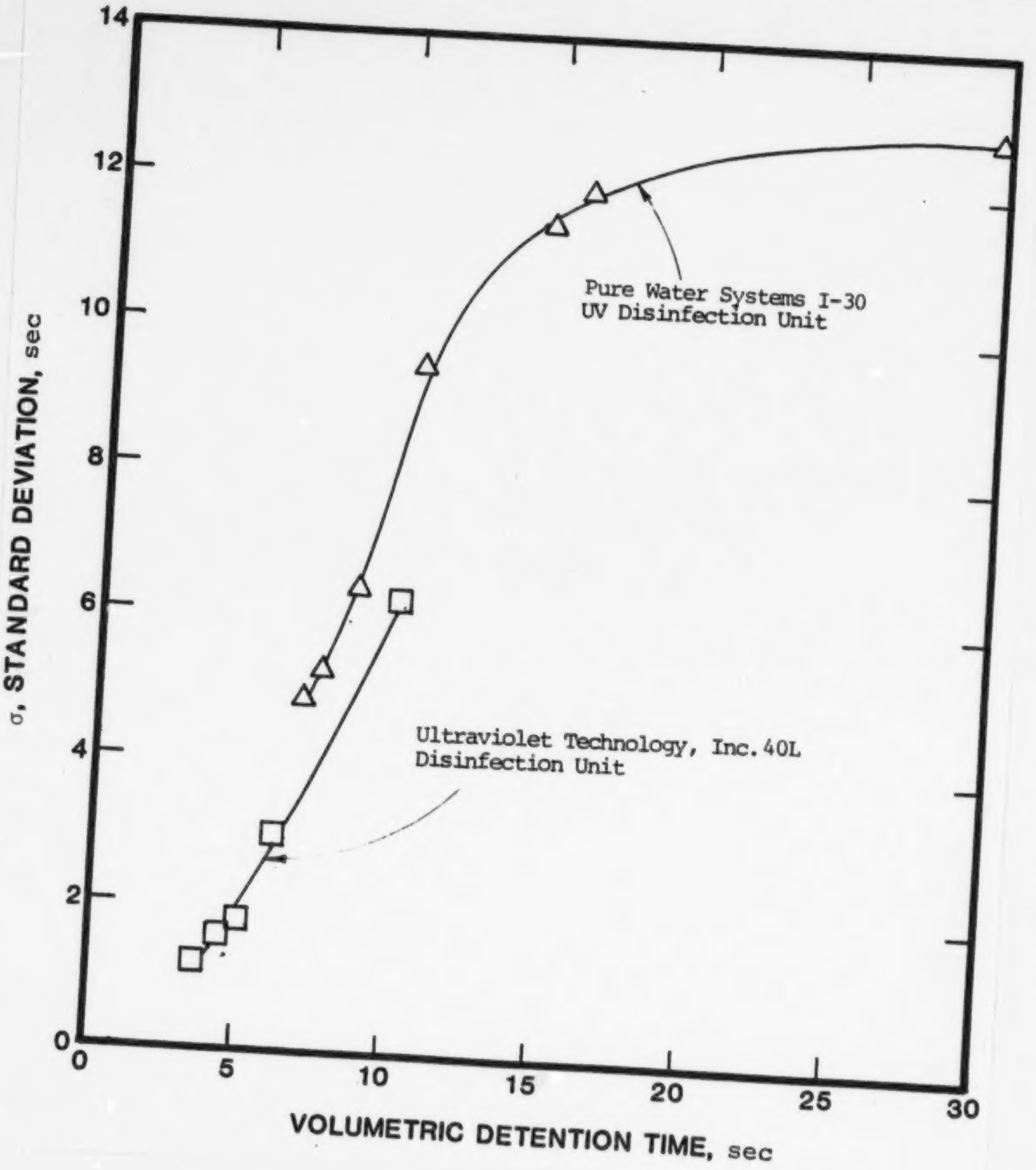
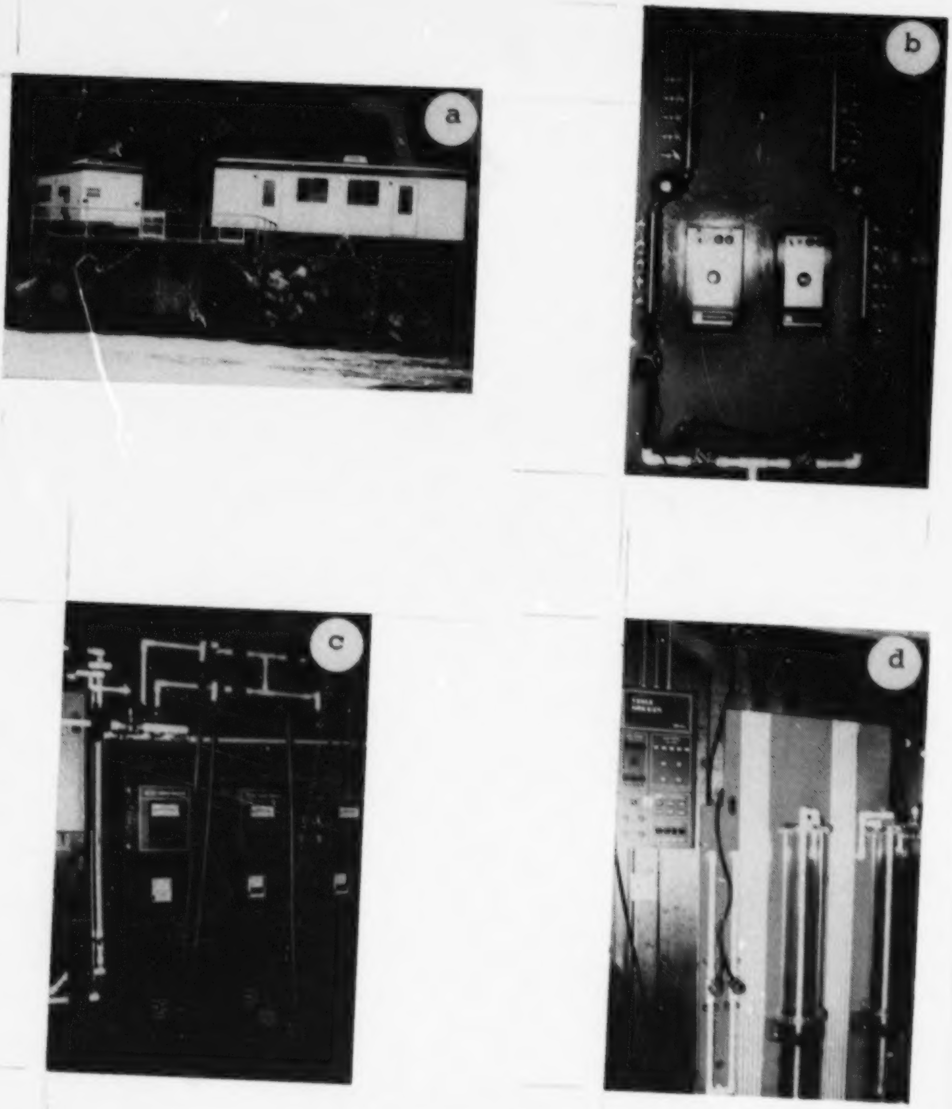


Figure 15. Flow characteristics of UV reactors as measured by standard deviation of RTD curve vs. detention time.



a. field research station at Cedar River intake, Seattle water supply; b. pump controls; c. pilot plant turbidity and flow network instrumentation; and d. filter column manometer boards and control panel

Figure 16. Field research station installation.

During normal operation the theoretical detention time could thus be changed from 16 to 44 minutes.

If, in addition to manipulating the water depth in the flocculation compartments, the flow rate through the two treatment trains was also altered, the range of detention times could be further expanded while still maintaining a 9.8 m/hr (4 gpm/ft^2) filter loading rate. This was made possible by a set of solenoid valves which enabled the plant operator to choose between three different operating conditions: 1) normal side-by-side operation of the two treatment trains, 2) combining the water from both flocculation streams to one filter, and 3) splitting the flow from one treatment stream between the two filters.

The flocculator paddles were suspended from a frame atop the flocculator unit. Each set of paddles was driven by separate variable speed motors. Rotational speed of each flocculator paddle was controlled by varying (1) the motor speed and (2) the size of the sprockets. During the present study tapered flocculation was used for both treatment streams. This pattern of flocculation, however, could be changed by exchanging some of the sprockets.

Filter Columns--

The two mixed media filters were constructed from 15.2 cm (6 in.) ID plexiglas columns. The composition of the 91.4 cm (36 in.) deep filter media is shown in Table 3. Eleven piezometer tubes made of 0.16 cm (1/16 in.) ID teflon tubing were used to monitor headloss at 10 cm (4 in.) intervals throughout the depth of the filter.

TABLE 3. COMPOSITION OF MULTIMEDIA FILTERS

Filter Material*	Depth of Material in.	cm	Effective Size mm	Uniformity Coefficient
Anthracite MS-4	18	45.7	1.0-1.1	<1.7
Sand MS-6	9	22.9	0.42-0.55	<1.8
Fine Garnet MS-21	3	7.6	0.18-0.22	<2.3
Course Garnet MS-22	3	7.6	1.0-2.0	-
Support Garnet	3	7.6		-

*Filter material supplied by Neptune Microfloc, Corvallis, Oregon

The filters and the accompanying valves and piping were mounted on a board to allow the filter columns to be lowered through the floor of the trailer during plant operation and hoisted back inside the trailer for transportation. The reason for this design was to provide sufficient head to the filters with gravity flow.

Before reaching the filters, the flocculated water passed through the flow control tanks which also functioned as semi-constant head tanks. Each tank had a float that controlled a valve which regulated the amount of water a centrifugal pump on the filter effluent line could deliver to the treated water distribution system. As headloss through the filter increased, the water level in the flow control tank would also increase slightly. Consequently the valve would open to maintain a constant pressure differential across the pump, and hence a constant flow rate through the filter. The filter loading rate could be varied from 4.8 m/hr (2 gpm/ft^2) to a maximum of 33.6 m/hr (14 gpm/ft^2) when both flocculator streams were combined to the same filter.

Filtered water from the two filters could be routed in a variety of ways including through a flow sensor, through a flow control valve to waste, through a five port manifold for adding microorganisms, and through a static mixer. From there, the water could go to further treatment such as disinfection.

The turbidity of the raw water as well as the effluent from the two filters was monitored continuously by Hach Model 1720A turbidimeters (Hach Chemical Co., Loveland, CO) and recorded on Rustrak Model 288 recorders (Gulton Industries, Inc., East Greenwich, RI).

For this UV disinfection study it was not necessary to utilize the water treatment pilot plant as just described.

UV DOSIMETRY METHODOLOGY DEVELOPMENT

Ferrioxalate actinometry

During the course of this investigation efforts were made to obtain a UV disinfection unit with a self-contained dosimeter so the actual UV dosage could be read directly. These efforts were fruitless and it was necessary to rely on actinometry to gauge the output of the UV unit. Since the geometry of the unit, the flow patterns at various Reynolds numbers, the degree and intensity of reflection, the average distance between lamps, the penetration depth, the cleanliness of the liquid and of the UV lamp, the degree of short-circuiting, and the degree of light scattering all have potential effects on the dosages applied, photo actinometry was used to assess the UV light intensity of exposure.

Conventional radiation dosages are commonly reported as a flux measurement, energy/cross-sectional area. Flux measurements can be determined with a radiometer, a photosensitive membrane which reacts to energy flux due to thermal effects. However, as previously stated, in practical applications, the use of a radiometer is limited by the geometric design and physical scale of the contactor. Direct correlation of ferrioxalate actinometry with radiometer measurements makes it possible to indirectly use radiometer measurements as the dose measurements, which are expressed in units of mW-sec/cm^2. As most dosages are conventionally reported per unit area, it is convenient to report dosages determined from ferrioxalate actinometry in terms of per unit area rather than per unit volume. Dosages determined by ferrioxalate actinometry were correlated to radiometer measured energy fluxes using the experimental setup by Qualls and Johnson (1983) to establish the bioassay-dose relation.

Bacillus subtilis Spore Bioassay

The bioassay method developed by Qualls and Johnson (1983) was intended to determine UV dosage in a solution in which direct UV measurement using a radiometer was not possible. In this method, *B. subtilis* (ATCC 6633) was used as an indicator organism in order to relate the degree of spore inactivation to the UV dosage applied to the spore suspension. A calibration curve depicting such a relationship as established by Qualls and Johnson (1983) is shown in Figure 17. By using the curve, UV dosage with respect to the log survival of the spores could be assessed, which in turn could be used to determine the intensity of the UV light through the reaction chamber.

CONTACTOR UV DOSE MEASUREMENT

Pen Ray UV Unit

Bioassay--

The Pen Ray UV unit was used as a UV source for batch studies of UV disinfection. The dimensions of the reactor are given in Figure 9. Pure cultures of *B. subtilis* spores (ATCC 6633) were diluted to 1 L with buffered water at room temperature to attain an initial spore concentration of approximately 10^6 cells/mL. A mixer was used to achieve a completely mixed condition in the spore suspension. The Pen Ray unit generated UV rays for irradiating the spore suspension. At different exposure times, water samples were taken from the reactor, using a 1 mL serological pipette, and diluted in buffered dilution water. Viable concentrations of the spores were determined by a standard plate count (Standard Methods, 1975).

Two bioassay tests were run. The log of survival rates of *B. subtilis* with UV exposure for the first experiment are presented in Table 4a. Data from Table 4a shows that after an exposure time of 5 minutes, spore survival dropped at a decreasing rate, implying that cell concentration became a limiting factor of the die-off rate. To avoid this limiting phase, the second run was designed to describe the die-off curve within the first five minutes of UV exposure. Table 4b shows data for the second run. Percent survival (Y) obtained from both experiments during the UV limiting period plotted with exposure time in minutes (X) can be expressed by the equation $\log Y = -0.916 X + 3.032$

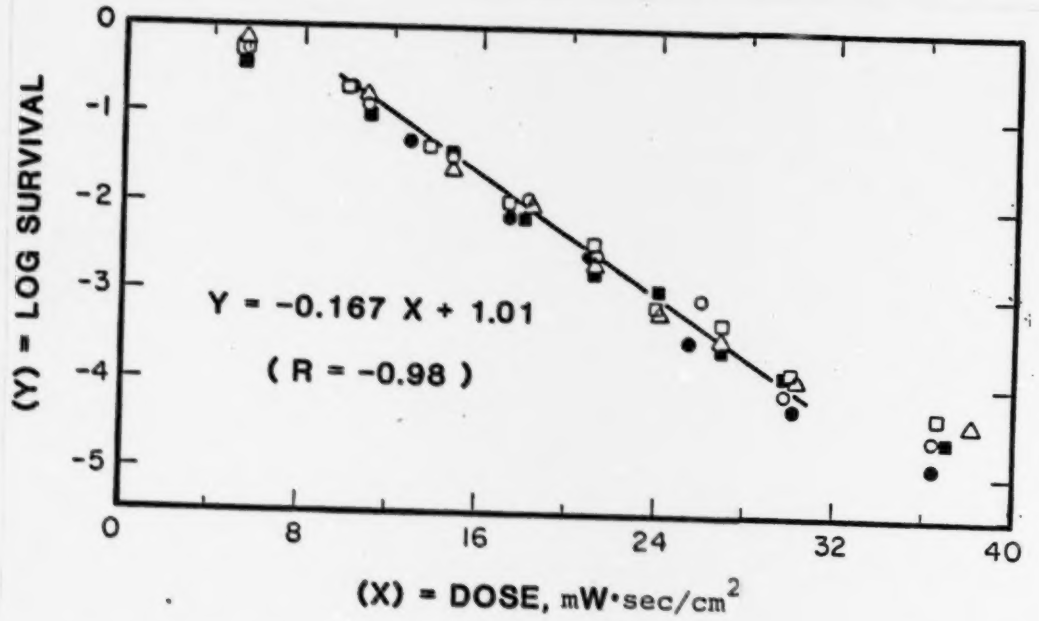


Figure 17. Calibration curve, Bacillus subtilis spore survival vs. UV dose.

with a significant degree of correlation of -0.99. Figure 18 shows this curve of _B. subtilis_ spores. The slope of the straight line depicts the log inactivation rate in the Pen Ray Batch Contactor due to UV irradiation. Using the die-off curve of _B. subtilis_ with the UV dosage relationship developed by Qualls and Johnson (1983), the average intensity of the Pen Ray unit was determined to be 0.0914 mW/cm^2. Die-off curves presented as percent survival versus exposure time for the Pen Ray can be altered to show percent survival versus UV dosage by simply multiplying the exposure time by the bioassay-determined intensity, 0.0914 mW/cm^2.

TABLE 4a. SURVIVAL OF _BACILLUS SUBTILIS_ (ATCC 6633) WITH EXPOSURE TIME TO UV FROM PEN RAY BENCH SCALE UNIT

Time (min)	No. of Organisms/mL	Log Survival
0	2.80×10^6	
2	3.75×10^5	-0.873
5	7.50×10^2	-3.572
8	3.75×10^2	-3.873
20	1.80×10^2	-4.192
30	1.20×10	-5.368

TABLE 4b. SURVIVAL OF _BACILLUS SUBTILIS_ (ATCC 6633) WITH EXPOSURE TIME TO UV FROM PEN RAY BENCH SCALE UNIT

Time (min)	No. of Organisms/mL	Log Survival
0	1.59×10^6	
1	1.26×10^6	-0.101
2	5.35×10^5	-0.473
3	4.60×10^4	-1.539
4	2.08×10^3	-2.883
5	5.15×10^2	-3.489

Pure Water Systems Unit (PWS Unit)

UV energy generation by the Pure Water Systems commercial UV unit was measured using the bioassay process described previously. With Seattle tap water, untreated with dechlorinating agent, as the carrier liquid, _B. subtilis_ spore suspensions were injected into the UV reactor at a flow rate of 3 mL/sec. After passage through the reactor, water samples were collected and split into two aliquots. To one aliquot, sodium thiosulfate (1.5 mL 10^{-6} M $Na_2S_2O_3$ per 20 mL) was added to eliminate residual chlorine. The other aliquot was unaltered. Table 5 indicates that spore counts of samples treated and untreated with sodium thiosulfate showed no significant difference.

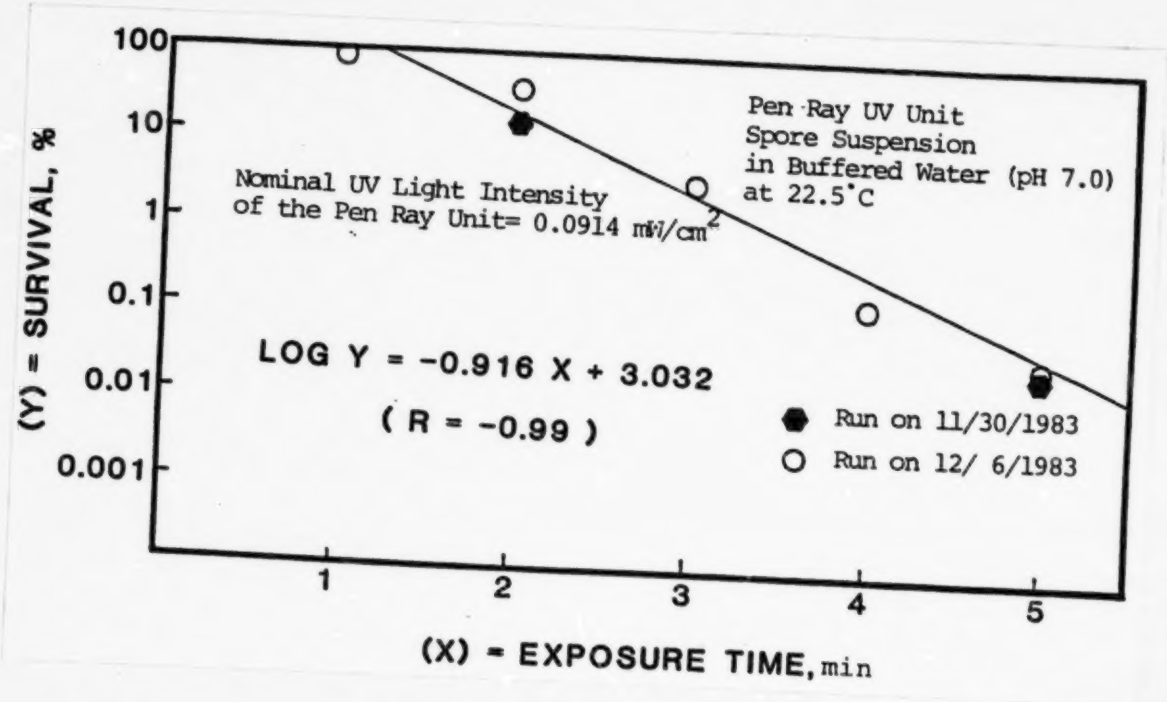


Figure 18. Percent survival of Bacillus subtilis vs. exposure time to UV generated from Pen Ray unit.

TABLE 5. BACILLUS SUBTILIS SPORE CONCENTRATION IN SAMPLES WITH AND WITHOUT ADDING SODIUM THIOSULFATE AFTER RUNNING THROUGH PURE WATER SYSTEMS UNIT

Theoretical Detention Time, sec	Concentration with $Na_2S_2O_3$, No./mL	Concentration without $Na_2S_2O_3$, No./L
28.6	9.46×10^2	9.90×10^2
15.1	2.97×10^3	2.82×10^3

Log spore reduction attained at varying detention times of the PWS unit is demonstrated in Table 6. Figure 19 shows the variation of receptor UV intensity and UV dosage with theoretical detention times. The intensity data were extrapolated to obtain a maximum average UV intensity of 4.350 mW/cm^2 at the intercept of Y axis.

TABLE 6. SURVIVAL OF BACILLUS SUBTILIS SPORES WITH INCREASING THEORETICAL DETENTION TIMES USING THE PURE WATER SYSTEMS UNIT

Theoretical Detention Time, sec	Concentration without UV No./mL	Concentration with UV No./mL	Log Survival	Calculated UV Intensity mW/cm^2
5.0	3.25×10^5	8.05×10^3	-1.61	3.14
10.2	9.59×10^5	4.37×10^3	-2.34	1.97
15.1	6.72×10^5	2.86×10^3	-2.37	1.34
28.6	8.22×10^5	9.46×10^2	-2.94	0.83
57.1	7.20×10^5	4.35×10^2	-3.22	0.44

Receptor UV intensities determined by the bioassay method at relatively high detention times were significantly lower than the maximum value. The inefficiency of the unit when used to perform the bioassay method with B. subtilis could be attributed to short-circuiting of the unit at low flow rates.

Ultraviolet Technology, Inc. Unit (UTI Unit)

UV output of the UTI unit was determined by the spore bioassay technique also. Table 7 shows the effect of increasing detention times on survival of B. subtilis spores in the UTI unit. The Ultraviolet Technology, Inc. unit's UV dosage curve shown in Figure 20, when extrapolated, yielded a maximum average receptor intensity of 14.4 mW/cm^2. If the dose curve is assumed to be linear at low theoretical detention times (<2.5 seconds), extrapolation of the intensity curve should be horizontal from the data point at 2.5 seconds, resulting in an intensity of about 8.8 mW/cm^2. The difference in the two intensity values indicates the limitation of the bioassay technique for determining receptor UV intensity.

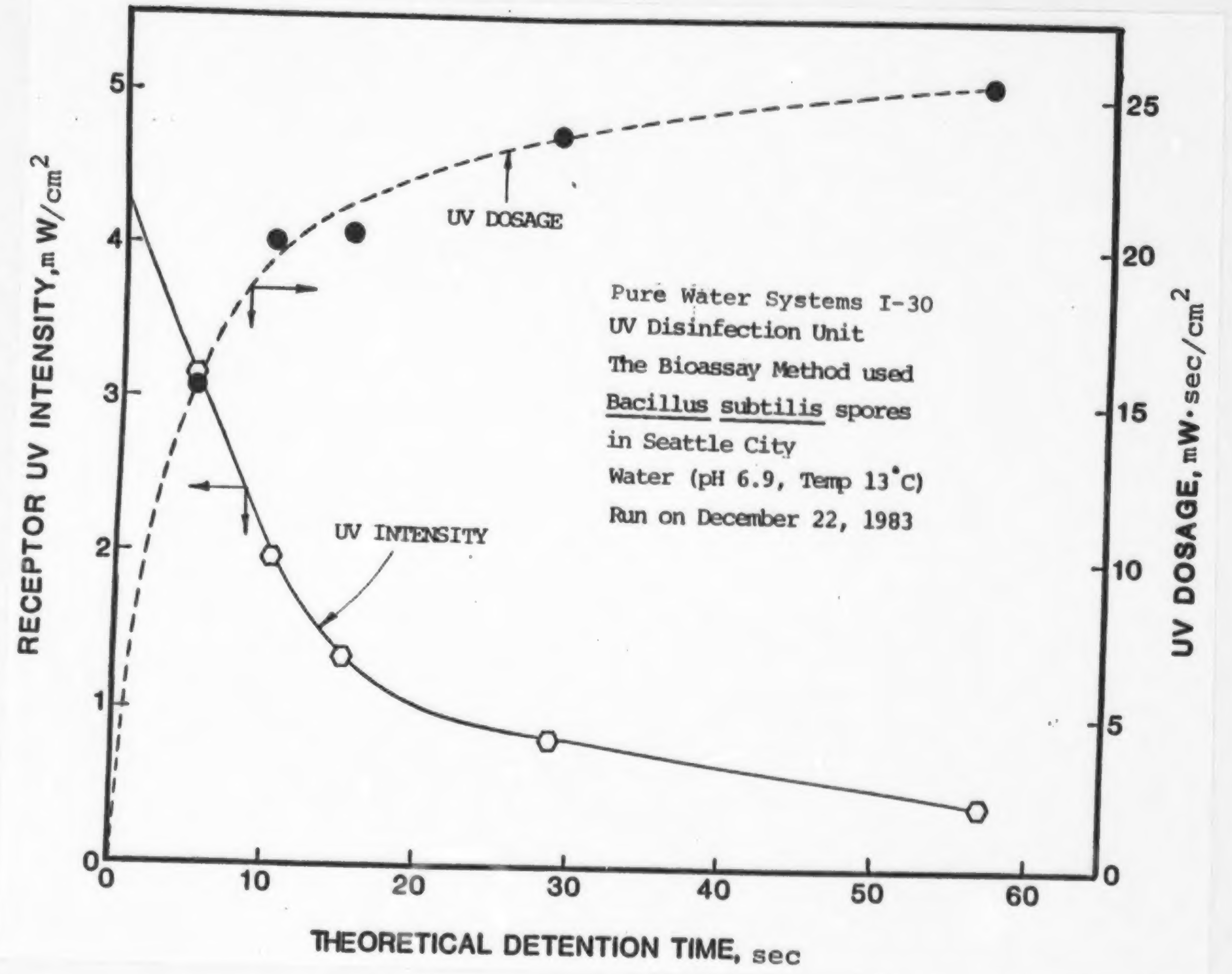


Figure 19. Variation of receptor UV intensity and dosage with theoretical detention time for UV reactor (Pure Water Systems, Inc.).

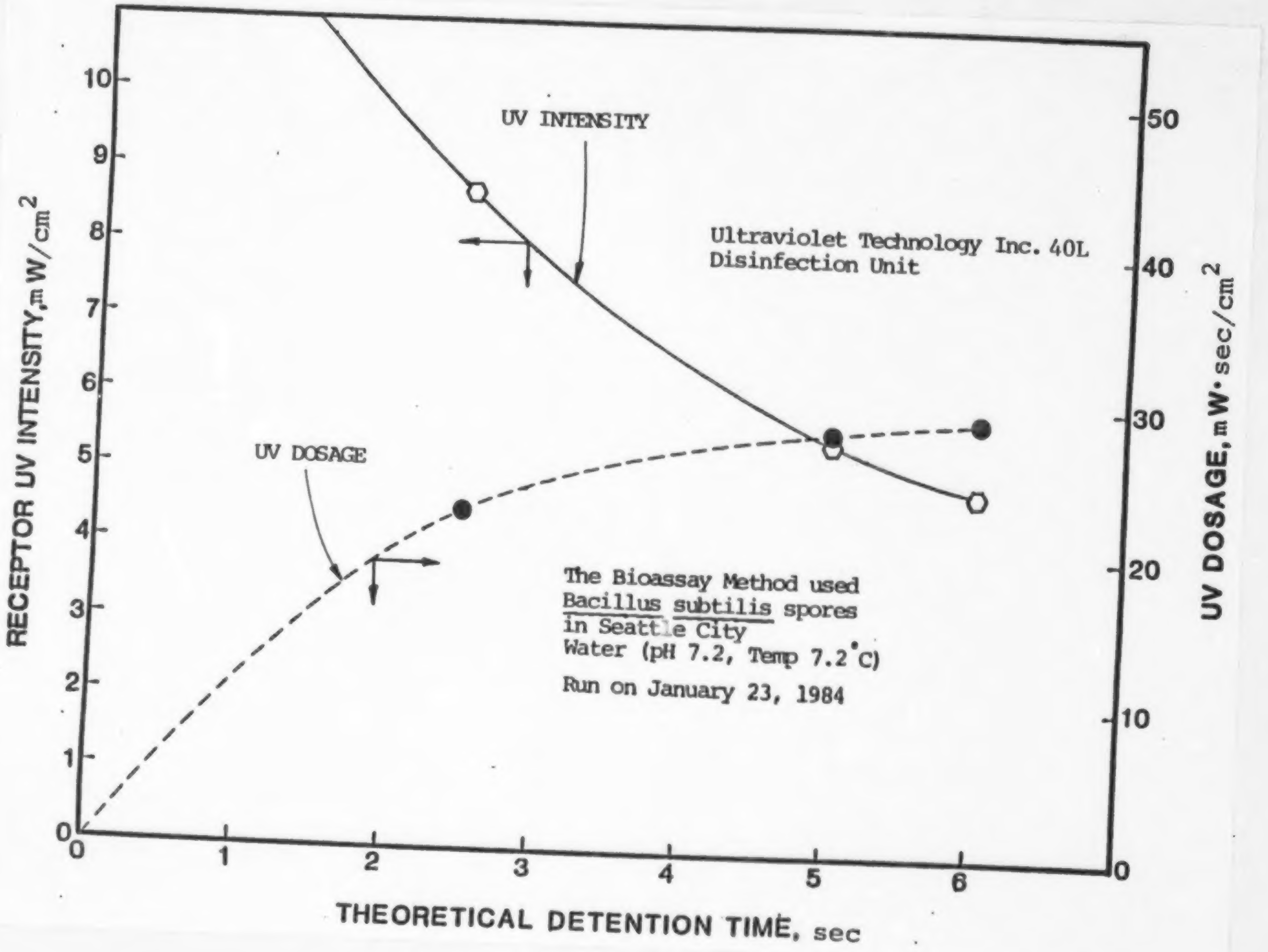


Figure 20. Variation of receptor UV intensity and dosage with theoretical detention time for Ultraviolet Technology unit.

TABLE 7. SURVIVAL OF BACILLUS SUBTILIS SPORES WITH INCREASING THEORETICAL DETENTION TIMES USING THE ULTRAVIOLET TECHNOLOGY UV UNIT.

Theoretical Detention Time, sec	Concentration without UV No./mL	Concentration with UV No./mL	Log Reduction	Calculated UV Intensity mW/cm^2
2.5*	7.56×10^6	3.18×10^3	2.690	8.86
3.8**	4.62×10^5	2.04×10^3	2.350	5.25
5.0*	1.38×10^6	3.95×10^2	3.543	5.45
6.0	8.85×10^5	1.44×10^2	3.788	4.79

*Temp. of water = 7.2°C, pH = 7.22, run on 1/23/84
**Temp. of water = 12.2°C, pH = 7.05, run on 12/22/83

Excimer Laser

Because of inconsistent intensity of each pulse produced from the excimer laser, the bioassay method could not measure the receptor UV intensity of the laser unit. Nevertheless, inactivation of *B. subtilis* spores after exposure to different laser output energies was determined. Table 8, as well as Figure 21, shows the results of *B. subtilis* spore survival versus UV energy generated by the excimer laser.

TABLE 8. SURVIVAL OF BACILLUS SUBTILIS SPORES AFTER EXPOSURE TO UV LIGHT FROM AN EXCIMER LASER

Output Energy from Laser, mW-sec (measured by sensor connected to oscilloscope)	Concentration after exposure, No./mL	Log Survival
272.6	8.6×10^5	-0.709
590.3	1.4×10^5	-1.497
857.6	4.0×10^4	-2.041
1227.2	1.5×10^4	-2.467
1760.3	1.1×10^4	-2.602
2357.8	2.0×10^3	-3.342

NOTES:
1. A 1 mL of sample was added into a quartz cell and exposed to UV radiation from the excimer laser. Sixty-three percent of the input energy was transmitted through the sample to the oscilloscope sensor.
2. Stock concentration 4.4×10^6 cells/mL.

UV DISINFECTION STUDIES

Giardia

Pen Ray Batch Reactor--

As shown in Figure 22 it can be seen that a relatively long exposure time was necessary to inactivate *G. muris* cysts in distilled water using the Pen Ray UV unit in a batch mode. Referring to Figure 23, an average exposure time of 20 minutes was required for a 99 percent kill of the cysts. By using this

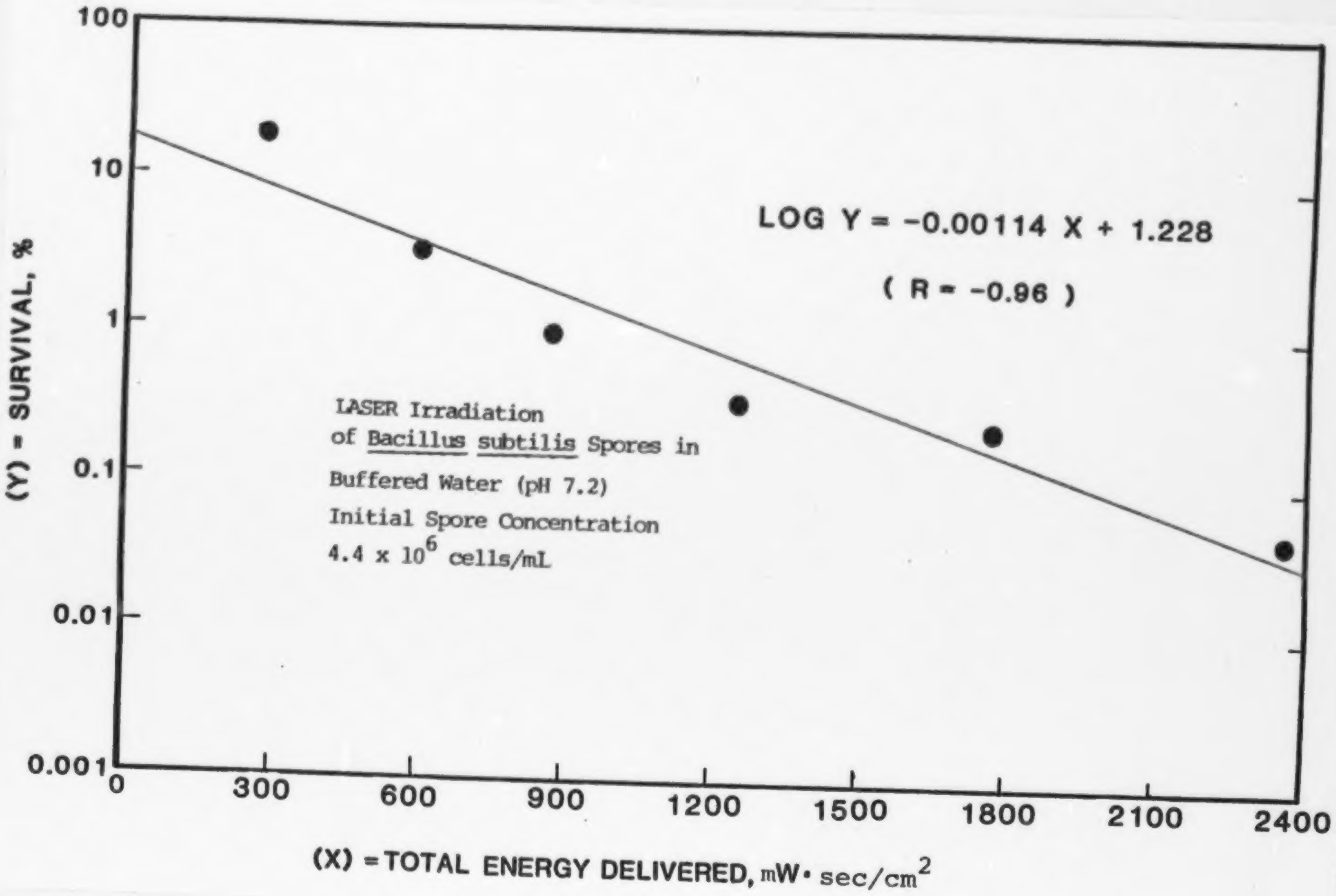


Figure 21. Inactivation of Bacillus subtilis (ATCC 6633) spores after laser generated UV irradiation.

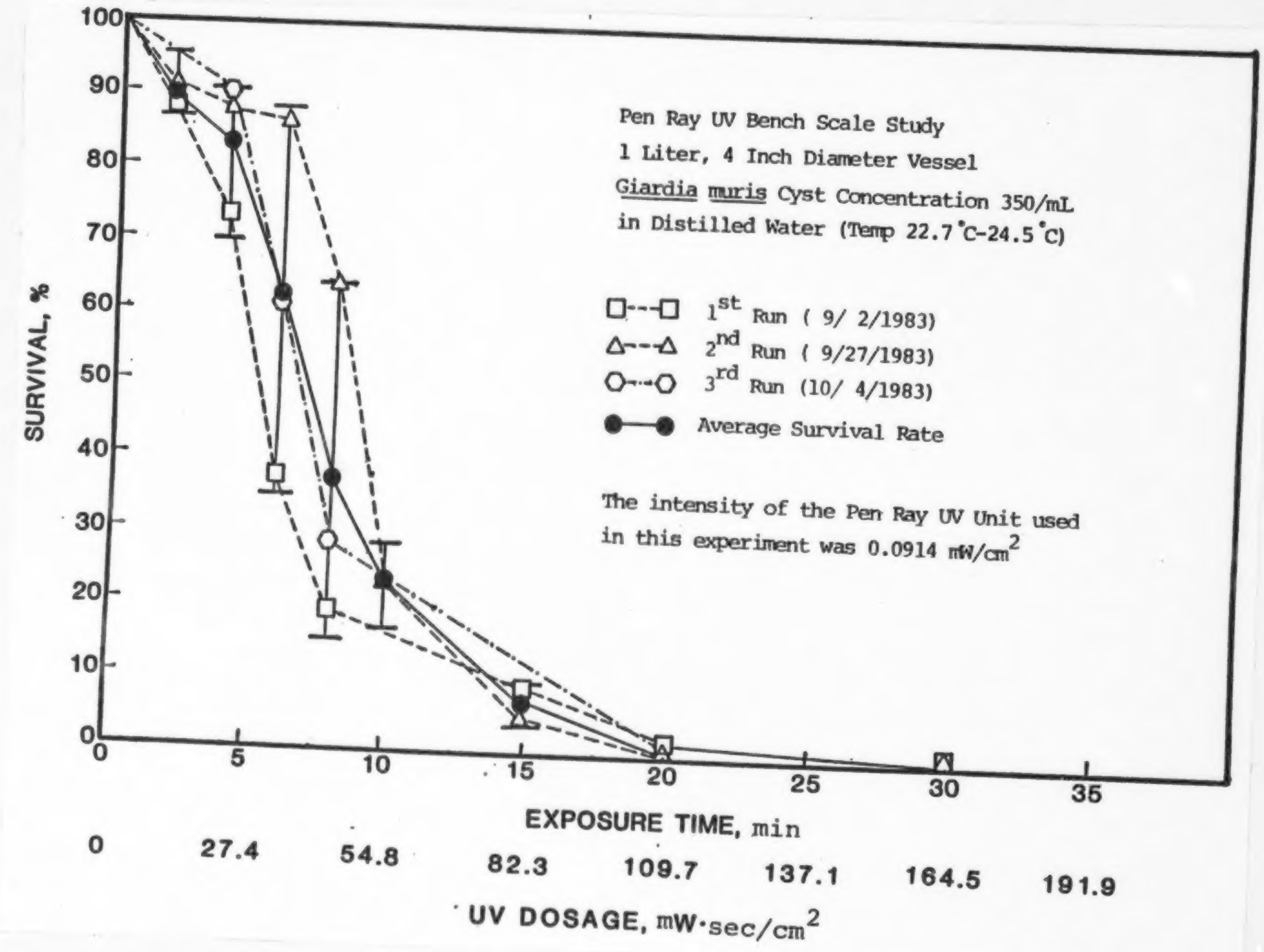


Figure 22. Giardia muris cyst survival vs. UV exposure time with a Pen Ray UV lamp.

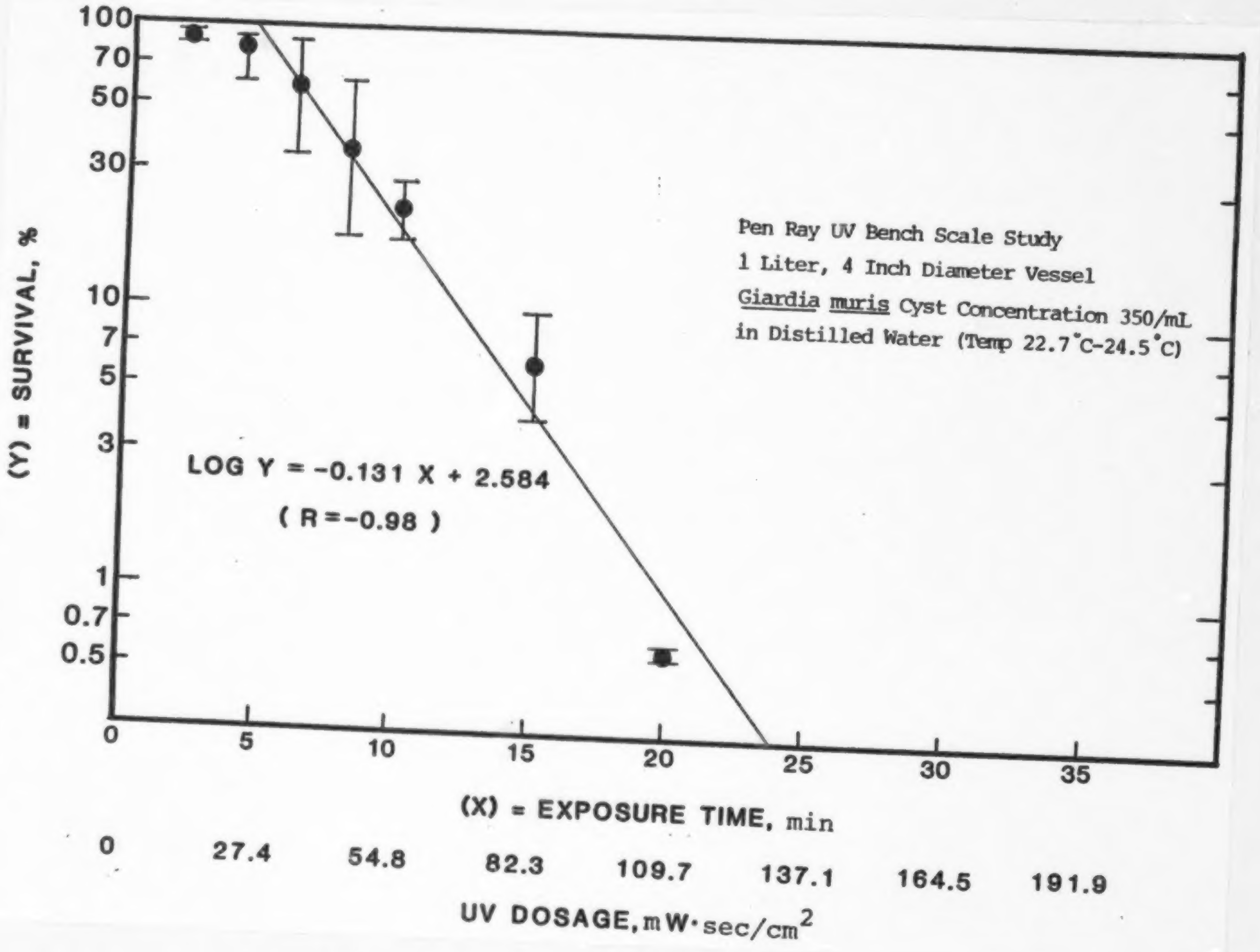


Figure 23. *Giardia muris* cyst survival vs. UV exposure time with a Pen Ray UV lamp (semi log plot).

information the UV dosage needed for a desired inactivation of G. muris cysts was known. These same data would be influenced by short-circuiting and flow regime in the flow through units.

Pure Water Systems Flow Through Unit--

Preliminary--Initially, a series of experiments were conducted to assess the limitations of the PWS-UV reactor with regard to total inactivation of G. muris cysts. For this reason, especially long detention times (high UV doses) were studied. Limitations of the internal volume of the reactor were such that a recycle system was utilized to obtain desired long detention times. The recycle system, consisting of necessary plumbing, a pump, a pressure vessel, and the UV reactor, resulted in a total test volume of six liters. A suspension of cysts in distilled water (concentration = 21 cysts/mL) was pumped into the recycle system, with effluent from the UV reactor recycled directly to the influent port. Detention times ranged from 4.5 to 30 minutes, resulting in corresponding UV doses of 3780 to 25,200 mW-sec/cm^2. At the start of each experiment, the UV lamps were allowed to warm up a sufficient time to reach a constant lamp temperature. Viability of the cysts was determined by excystation.

Giardia cysts were spiked into the UV influent stream through a manifold and mixed directly downstream by means of a static mixer. The cysts passed through the UV contactor only once, with the effluent, subsequently being collected, concentrated and excysted. Twelve detention times within the range of 5.4 to 120 seconds were studied. Reported detention times are theoretical volumetric detention times - volume divided by flow rate. The UV intensity was maintained at uniform power for all exposures.

Results of the inactivation study are shown in Figure 24, a semilog plot of cyst survival (percent) versus UV dose. Within the dose rate of 140 mW-sec/cm^2 (10 sec), the dose versus response curve closely resembles the shape of the batch curve, a small shoulder initially followed by a linear die away. About 98 percent of the cysts were inactivated with a 15 second detention time. Beyond this range, additional inactivation of Giardia cysts was difficult. Complete inactivation, i.e., 100 percent kill, was attained only for the 80 and 120 second detention times. These particular points are plotted as the percent survival indicating the statistical probability of one cyst survival (no survival was found by the testing procedures).

The persistence of only a 2 log inactivation up to detention times of 60 seconds is probably, among other things, a function of the hydraulic flow characteristics of the PWS-UV reactor. At volumetric detention times greater than 8.5 seconds, short-circuiting, as characterized by the standard deviation (σ^2) of residence time distribution curves, increases significantly.

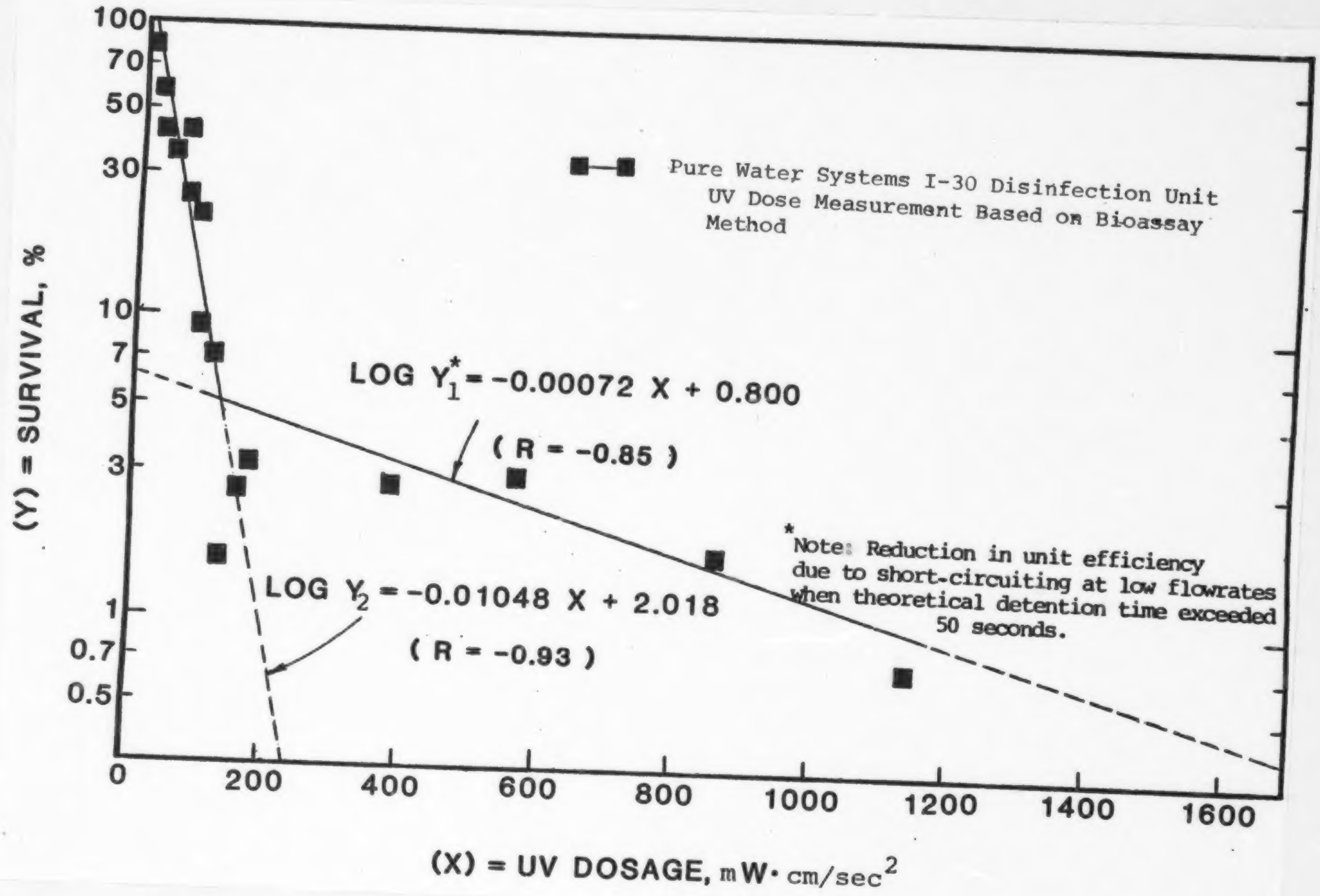


Figure 24. Giardia muris cyst survival vs. UV dosage for Pure Water Systems UV unit.

This rise in σ^2 with detention time finally begins to level off at detention times around 30 seconds. Reynold numbers also indicate that transition regions of mixing in the reactor occur in the detention time range of 15 to 17 seconds.

A comparison of *Giardia* cyst survival versus UV dosage from a study by Rice and Hoff (1982) and data obtained in this investigation is shown in Figure 25. It is significant to note that the relatively low destruction of *Giardia* cysts noted by Rice and Hoff was due to the low UV exposure dose. The two sets of data were then extrapolated to produce the curve as shown in Figure 25.

Color Addition--Two sources of color were used as UV absorbers: tannic acid and reverse osmosis concentrate of the Cedar River (ROCCR). The reverse osmosis unit was operated at 1.9 L per minute and 100 psi operating pressure using a polysulfone membrane with a characteristic molecular weight cutoff of 1000. Figure 26 presents representative spectral curves of Cedar River water, Cedar River water spiked with tannic acid, and Cedar River water spiked with ROCCR.

Tannic acid was selected as a color source because of its high selective absorbance at wavelengths around 254 nm, as well as its similarity to color naturally found in many water supply sources. The tannic acid solution was prepared from laboratory grade tannic acid and distilled water. The solution was neither filtered nor centrifuged. Unlike the tannic acid solution, the Cedar River water concentrate exhibited no specific absorbance peaks. Absorbance of the ROCCR gradually increased with a decrease in wavelength, down to 210 nm when water absorption then becomes significant. ROCCR contained a high concentration of ions and particulates. Most of the particles were removed through centrifugation at 2500 γ and subsequent filtration through a 0.45 μ filter. For all three curves, the absorption dramatically increased at wavelengths smaller than 210 nm because of the absorption of water.

Under normal flow conditions, the Cedar River has a low concentration of absorbing material, as illustrated in Figure 26. Background data for the Cedar River recorded an average absorbance at 254 nm of 0.004/cm. During periods of intense rainfall and heavy runoff, absorbance of the Cedar River water increased to values slightly higher than 0.20/cm. For both low and high flow conditions, the shape of the spectral curves were similar. The curves differed only in vertical displacement with the high flow showing a greater absorbance.

Analysis of the inactivation curves for the color spikes is difficult because of problems encountered (during June and early July of 1983) with entrapped gas within the UV-reactor. Presence of the gas in the reactor decreased the hydraulic detention time

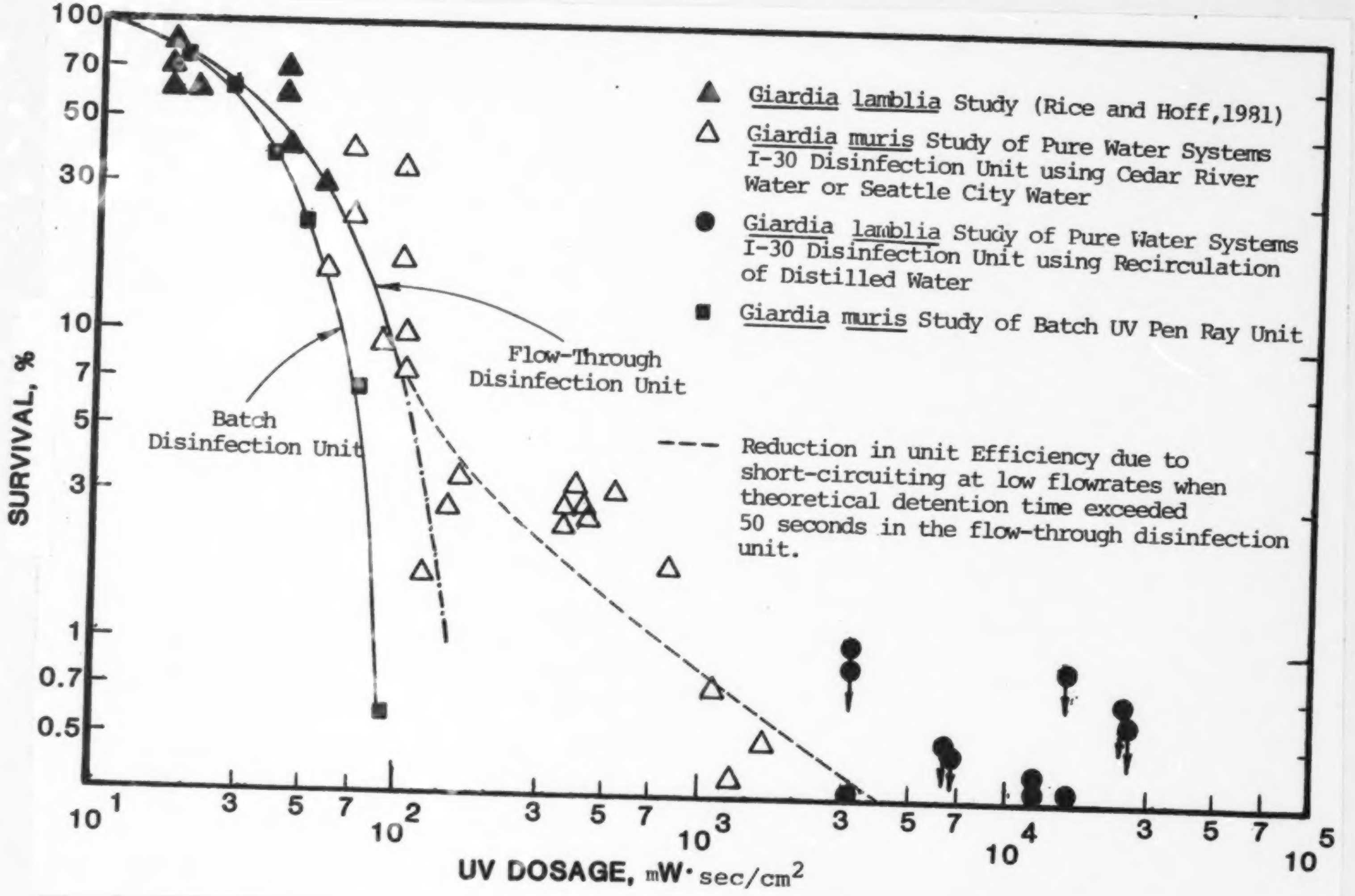


Figure 25. Survival of Giardia sp. cysts vs. UV dosage.

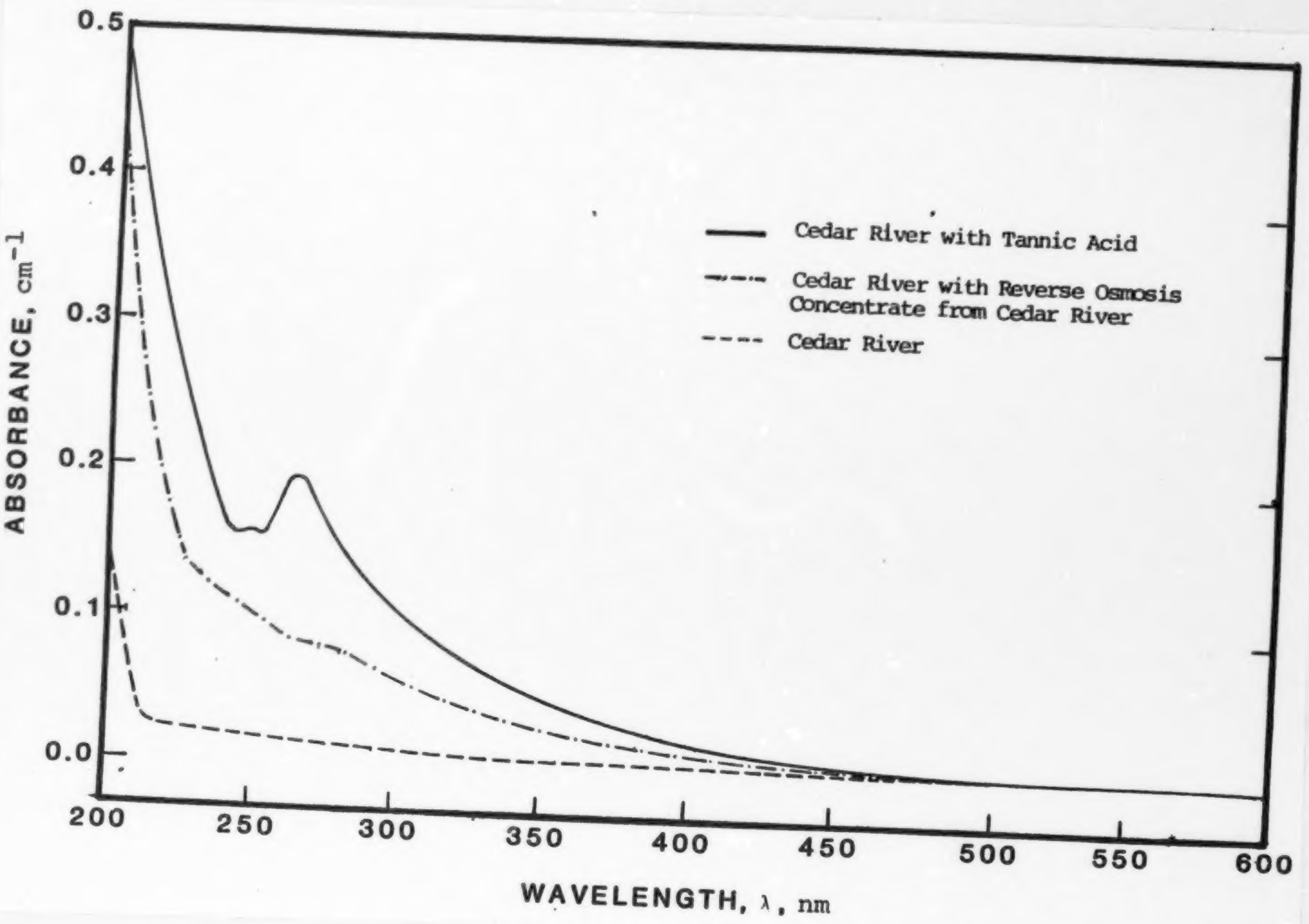


Figure 26. Spectral curves for Cedar River water with color additions from Cedar River reverse osmosis concentrate and tannic acid.

and probably increased short-circuiting. Super-saturated oxygen concentrations in the Cedar River and horizontally directed flow through the reactor probably contributed to the problems produced by the trapped gas. When this problem was discovered, the UV-reactor (PWS) was fitted with an air valve through which trapped gas could be released. Once in place, special care was taken to bleed trapped gas from the reactor before each experiment.

Results from the tannic acid versus cyst-inactivation studies are presented in Figure 27. Cyst-inactivation studies using reverse osmosis concentrate of the Cedar River are presented in Figure 28. Theoretical detention times at 10, 15, and 30 seconds were utilized for these experiments. Absorbance at 254 nm ranged from naturally occurring conditions of 0.003/cm to artificially inflated values of 0.34/cm produced by the color spikes. These same data are plotted slightly differently in Figures 29 and 30. Table 9 presents some basic water characteristics for these experiments.

For all experimental conditions, regardless of the source of color, an increase in absorbance at 254 nm resulted in an increase in the percentage of cyst survival. Significant increases were found for all four experiments using tannic acid. A theoretical retention time of 10 seconds resulted in a rise in cyst survival from 25 to 50 percent when absorbance values increased from 0.05/cm to 0.25/cm. Similarly, for a retention time of 30 seconds, as absorbance values increased from 0.01/cm to 0.16/cm, cyst survival increased from 5 to 16 percent.

In comparison, a 30 second retention time using the ROCCR-A (June and early July) spike resulted in the same degree of cyst inactivation as for the tannic acid spike. Cedar River, without the color spike (A = 254 nm), had a 1.7 percent cyst survival while the ROCCR-A run at an absorbance of 0.11/cm resulted in 10.2 percent cyst survival. The ROCCR-B spike, undertaken approximately four months after ROCCR-A runs, resulted in a higher survival rate, for a 30 second retention time, than values obtained during the tannic acid or ROCCR-A runs. Irradiation of the Cedar River under natural conditions (A = 0.04/cm) did not inactivate 9 percent of the original cyst spike. When the absorbance was increased to 0.18/cm using ROCCR, cyst survival increased to 18.5 percent. For the ROCCR-B run at a detention time of 15 seconds, cyst survival increased from 17.2 to 33 percent as absorbance values increased from 0.062/cm to 0.133/cm.

Comparison of the inactivation curves for both spikes can best be performed between the runs made at a detention time of 30 seconds. Survival curves using a tannic acid spike were quite similar to the curve resulting from ROCCR-A run. However, the log survival obtained for ROCCR-B appeared to be slightly higher than the other 30 second runs. Different ROCCR concentrates were used for ROCCR-A and ROCCR-B runs, and this could account for the

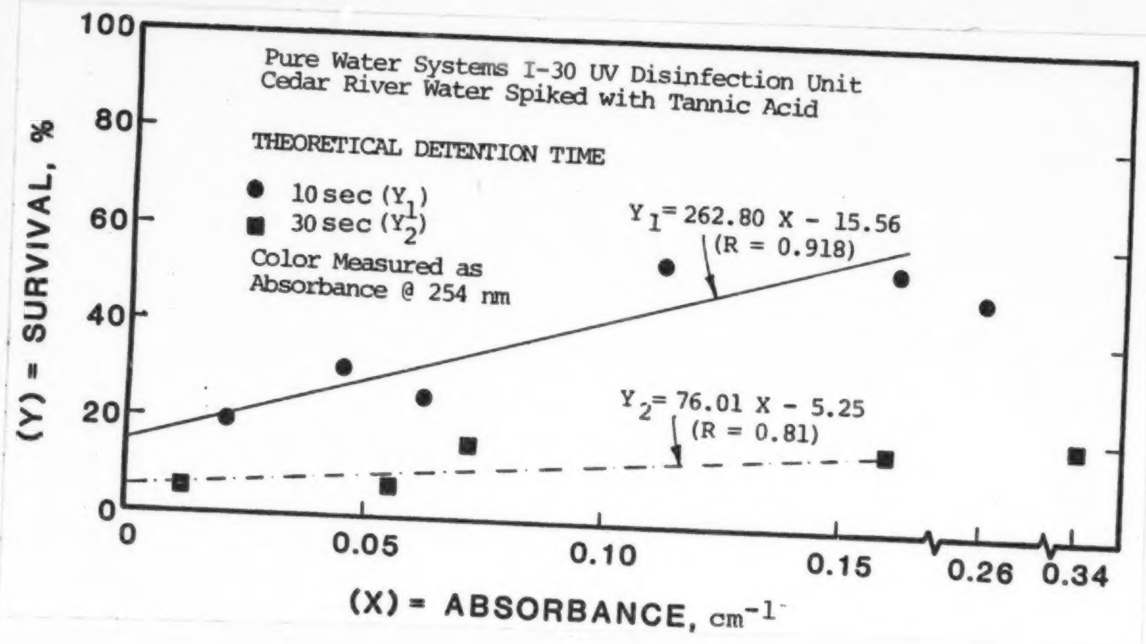


Figure 27. Effect of tannic acid color on survivability of _Giardia_ _muris_ cysts subjected to UV.

TABLE 9. WATER QUALITY CHARACTERISTICS OF CEDAR RIVER WATER FOR GIARDIA MURIS CYST INACTIVATION RUNS WITH COLOR SPIKES, PURE WATER SYSTEMS UV REACTOR

Run	Absorbance at 254 nm (cm-1)	Turbidity (NTU)	pH	T (°C)	Conductivity (µmhos/cm)	D.O. (mgO_2/L)	Total Alkalinity ($mgCaCO_3/L$)	Total Hardness ($mgCaCO_3/L$)	Giardia Conc. (cysts/mL)
Tannic Acid									
6/29/83	0.02 -0.16	0.21-0.32	7.2	14.5-16	40-47	8.6	20.0-22.0	26.2-27.6	296,330
10 sec									
30 sec									
7/6/83	0.003-0.337	0.19-0.26	7.2	14.2-16.4	39-48	8.6	22.2-24.6	22.6-27.6	190,2 2
10 sec									
30 sec									
ROCCR*									
8/17/83	0.007- .118	0.43-1.1	7.2-8.0	15.1-17.8	63-165**	-	35.5-76.0**	24.8-89.6**	310,332,264
10 sec									
15 sec									
30 sec									
12/1/83	0.47 -0.184	0.4 -0.65	6.7-7.2	10.7-12.0	24-97**	10.2-12.0	11.4-66.4**	14.6-76.0**	243,298

*Reverse Osmosis Concentrate for Cedar River Water

**ROCCR spike raised the total alkalinity, total hardness, and conductivity of Cedar River

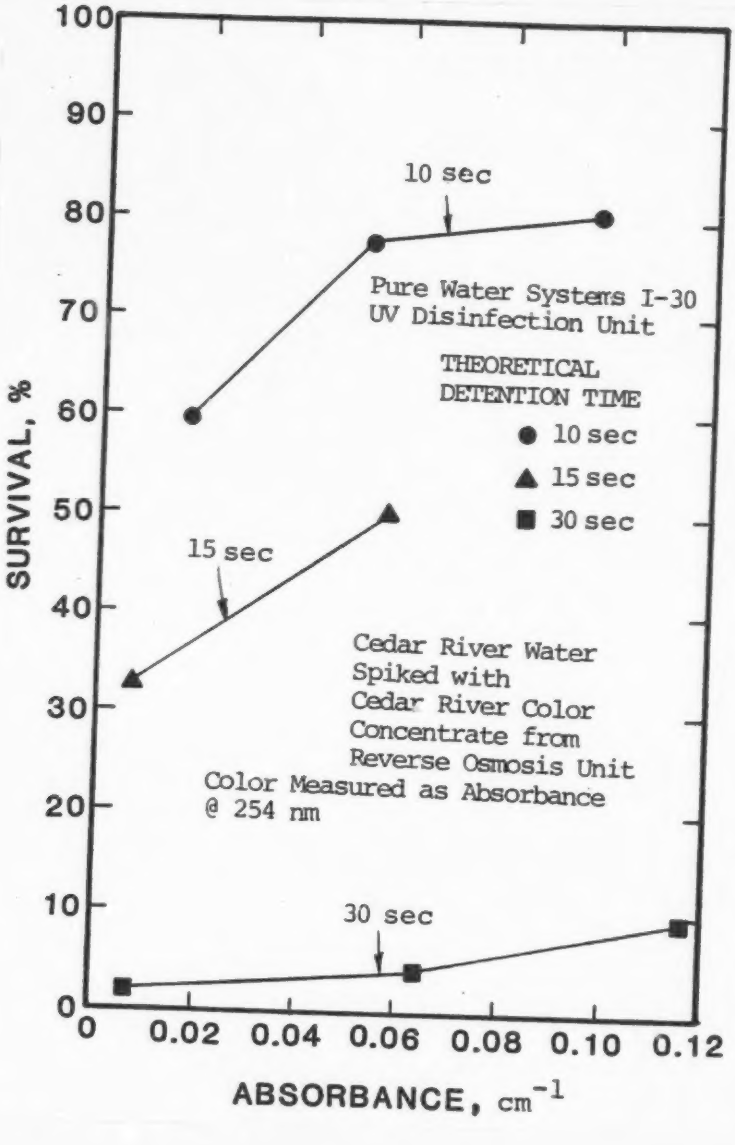


Figure 28. Effect of Cedar River color on Giardia muris cyst survival for detention times under UV light of 10, 15, and 30 seconds duration.

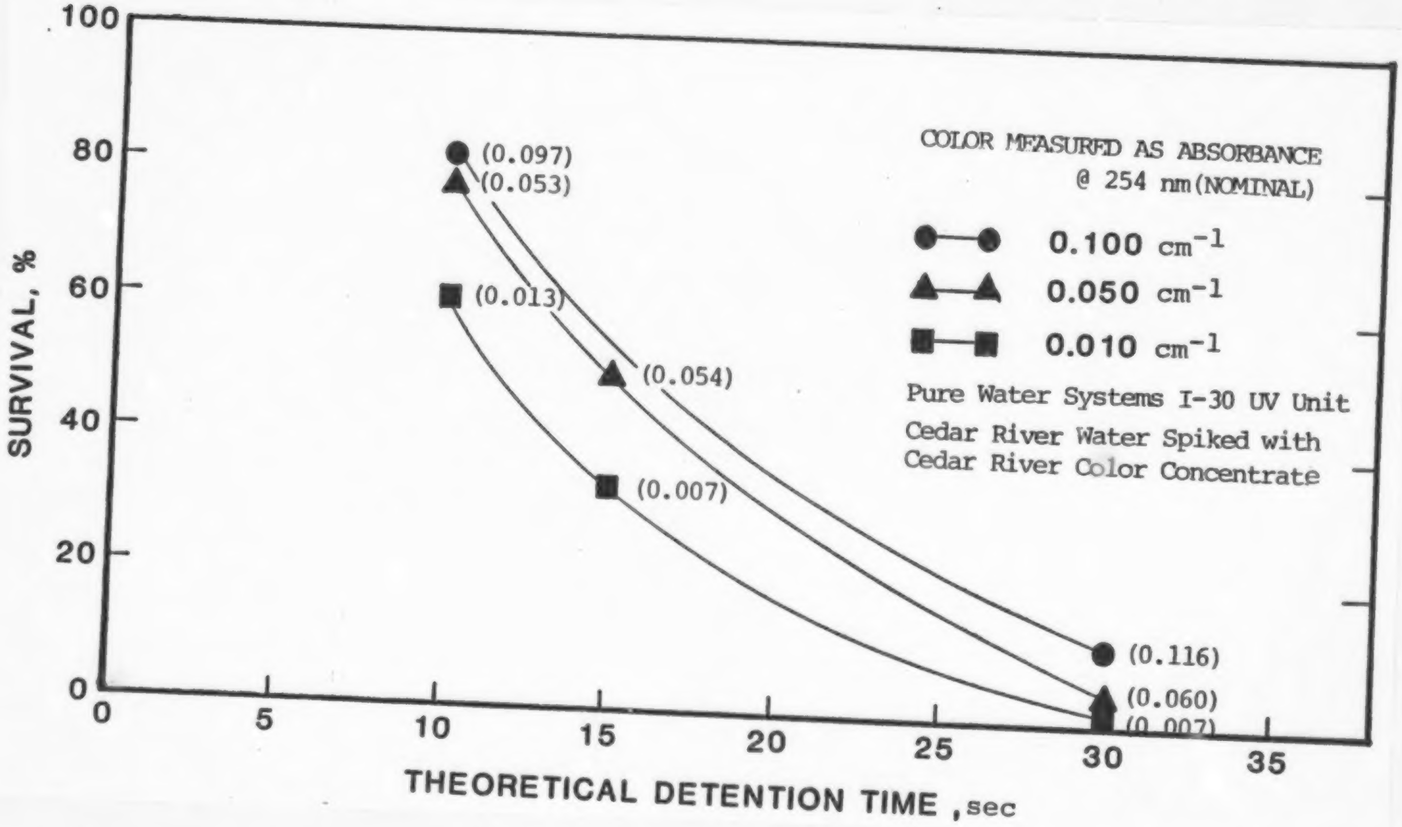


Figure 29. Effect of Cedar River color on *Giardia muris* cyst survival under UV light at various detention times for Pure Water Systems unit.

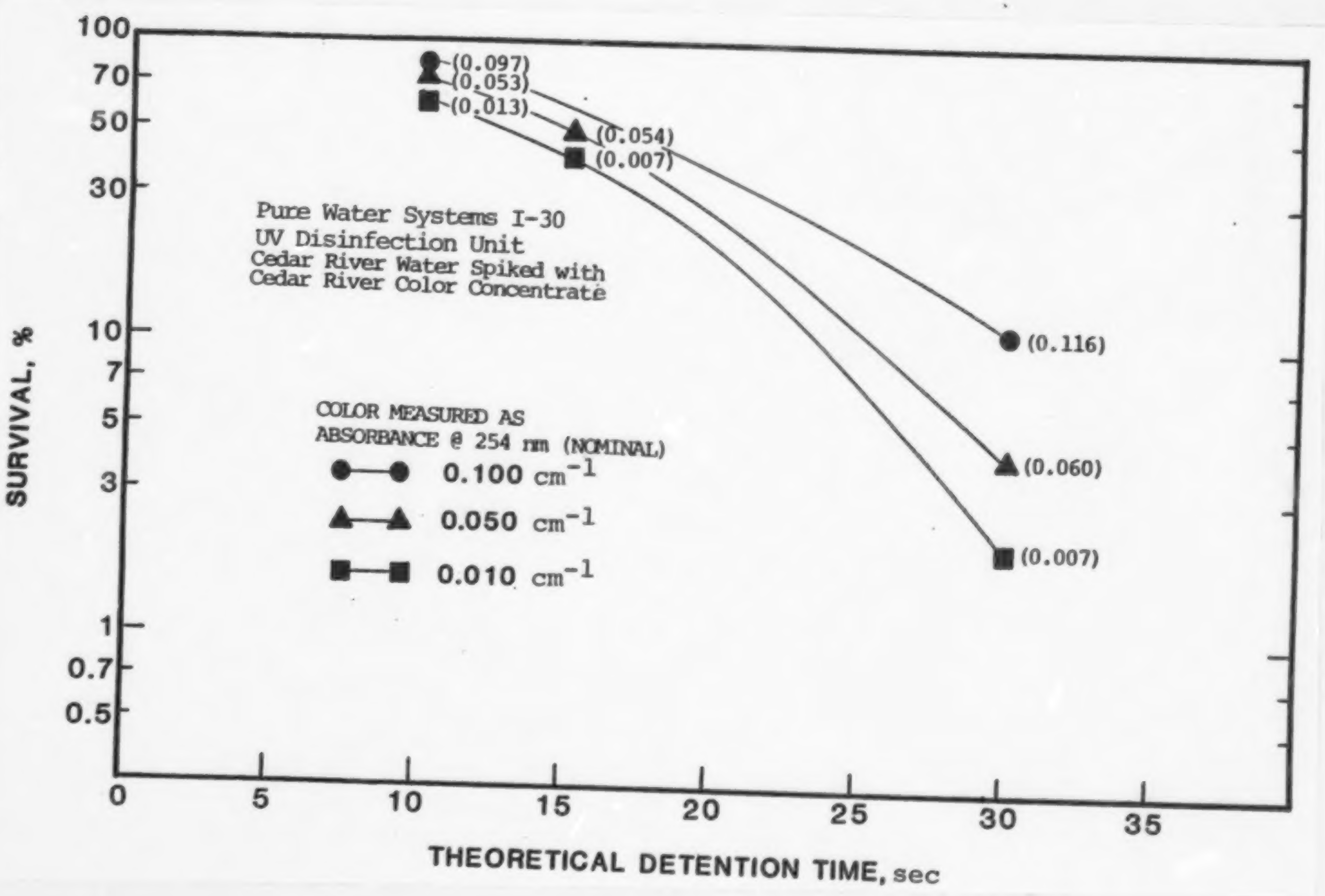


Figure 30. Effect of Cedar River color on *Giardia muris* cyst survival under UV light at various detention times for Pure Water Systems unit.

differences in the survival curves. However, the spectral curves of the filtered solutions for both ROCCR solutions were fairly similar. Explanations for the perceived difference in cyst survival between the ROCCR-A and ROCCR-B runs can then be narrowed to 1) the degree of scatter and refraction (a result of particulates), and 2) a decrease in the intensity of light reaching the microorganisms, either due to a decay in lamp output or a reduction in the transmittance of the quartz sleeve.

The Beer-Lambert law can be used to approximate the average received UV dose in a reactor if the effects of scattering and refraction are discounted. The transmittance, τ, is defined as I/I_O where I_O is the initial beam intensity which is reduced to I after passage through a layer of thickness, b. Absorbance, A, is defined as $A = eMb$ where e is the extinction coefficient, M is the molar concentration of the fluid and b is the thickness of the fluid layer in centimeters (Phillips, 1983). Then $A = -\log \tau$.

Difficulty in this analysis resides in the ability to accurately measure the initial beam intensity (I_O) and to determine b, the travel distance of UV light through the media. According to the actual plans of the UV reactor, the average distance from a quartz sleeve to the outer wall is 1.27 cm. However, the outer shell of the reactor is highly reflective, which increases the distance of light travel in excess of a single passage through the physical dimensions of the reactor. Using data from the ROCCR-B run, an attempt was made to back calculate b from absorbance values and UV doses as measured by the bioassay. Filtered absorbance values were used for calculating b. Using the cyst survival values for the detention time of 30 seconds and an $I_O = 3.6$ mW/cm^2 based on manufacturer's claims, an average b value of 3.0 cm was found.

Turbidity--As with the color spikes, two sources of turbidity were used for the turbidity spiked Cedar River water experiments: inorganic turbidity from the White River and organic turbidity from the reverse osmosis concentrate of the Cedar River (ROCCR).

Samples of inorganic turbidity were obtained from the White River which originates on Mount Rainier, flows down the east side of the mountain, winds around to the north, and then westerly to its confluence with the Puyallup River at Puyallup, Washington. The river gets its name because of its appearance, a result of the heavy burden of glacial till particulates the river carries.

Samples of White River water were prepared for disinfection runs by settling the sample for three days and decanting the supernatant. This settling and decanting procedure was repeated with the supernatant. This second supernatant was then used for spiking UV disinfection runs.

Initial runs which were made with White River turbidity created a significant problem during cyst excystations. Particles present in the samples masked the cysts and trophozoites, making visual analysis of the excystation virtually impossible. To overcome this problem, the White River turbidity spike was filtered through a five µm cartridge filter. Particles five µm in size or less should pass through the 293 mm nucleopore filter used for concentrating the Giardia cysts and therefore, should not be present in the samples concentrated for excystation.

The organic source of turbidity was obtained from the ROCCR. Particles were removed from the ROCCR by centrifugation at 2500γ and subsequent filtration through a 0.45 µm membrane filter. After resuspension in distilled water, the particulate suspension was passed through a five µm nucleopore filter to reduce problems which the particles may create for cyst excystations. The problems of dirty cyst samples experienced with the filtered White River spikes were not as prevalent with the filtered ROCCR turbidity spike. Characteristic particle size distributions for Cedar River spiked with turbidity are displayed in Table 10.

TABLE 10. PARTICLE SIZE DISTRIBUTION FOR CEDAR RIVER WATER SPIKED WITH WHITE RIVER OR REVERSE OSMOSIS CONCENTRATE FROM CEDAR RIVER PARTICULATES (ROCCR)

Sample	Particle density, No. /mL			
	40-20µm	20-10µm	10-5µm	5-2.5µm
White River				
Run 1* - 8.3 NTU	142	808	6621	19908
0.3 NTU	70	131	1730	6812
Run 2 -10.0 NTU	29	365	1084	7151
0.2 NTU	30	319	806	3722
ROCCR				
Run 1 - 8.5 NTU	44	168	1402	15265
1.7 NTU	34	195	1191	14682

*Some entrapped gas noted in reactor

Filtering of the White River turbidity did not remove all turbidity interference. From time to time, concentrated Giardia samples still required cleaning before an excystation could be performed. This involved stirring the concentrated cyst sample, letting the sample sit quiescently for a period of time, usually 30 minutes, and withdrawing the supernatant for use in the excystation. After cleaning, both the supernatant and subnatant were examined microscopically to make certain that (1) cysts were present in the supernatant and that (2) the supernatant was free of particles which might interfere with visual analysis of the excystations. If the two conditions were not satisfied, the supernatant was reunited with its subnatant and the cleaning procedure was repeated.

Turbidity spikes were introduced into the flow stream (Cedar River) through a one-half inch manifold directly upstream from the UV reactor. The spike was injected by means of a Fluid Metering, Inc. pump (FMI pump). Injection of the turbidity was initiated several seconds before the pumping of the cysts in order to achieve a steady-state density of particles in the flow stream. Desired turbidities were obtained by varying both the feed rate of the FMI pump and the dilution of the stock turbidity solution. Turbidity stock solutions were diluted with Cedar River water. Turbidity values for the runs with White River particulates ranged from 0.2 NTU (at natural conditions) to 4.7 NTU for 10 seconds, 6 NTU for 15 seconds, 13 NTU for 30 seconds, and 10 NTU for 80 seconds. Turbidity values using ROCCR particulates ranged from an unspiked value of 0.2 NTU up to 4.7 NTU for a retention time of 10 seconds. For 30 seconds, turbidities ranged from 0.2 NTU to 13.0 NTU. Higher values could not be obtained because of the limitations of the pumping system and the concentration of the stock turbidity solution.

General evaluation of the UV cyst inactivation runs showed that longer detention times, i.e., larger UV doses, resulted in greater levels of inactivation. However, the levels of inactivation did not remain constant from one run to the next. This inconsistency does not appear to be due to the different sources or concentration of particles, but rather due to entrapped gas in the UV reactor (White River-Run 1) and the variability of the excystation technique (ROCCR-Run 1).

Specifically, comparison of cyst inactivations at varying turbidity showed that the presence of particles had no discernible effect on the UV inactivation of the cysts. Results also indicated that there was no difference in effect caused by inorganic or organic particles as the source of turbidity.

Figures 31 and 32 present the results of cyst inactivation runs using White River turbidity. The set of runs represented in Figure 31 was characterized by the presence of entrapped gas. Subsequently, the series of runs shown in Figure 32 represents experiments in which air was bled from the UV reactor before each inactivation test. Results of the cyst inactivation runs using ROCCR particulates for turbidity spikes are displayed in Figure 33. For all runs, varying turbidity while holding the retention time constant did not affect the degree of cyst inactivation. This was true even though the turbidity spike raised the absorbance of the Cedar River a small degree. Water quality characteristics for all runs with a turbidity spike are located in Table 11.

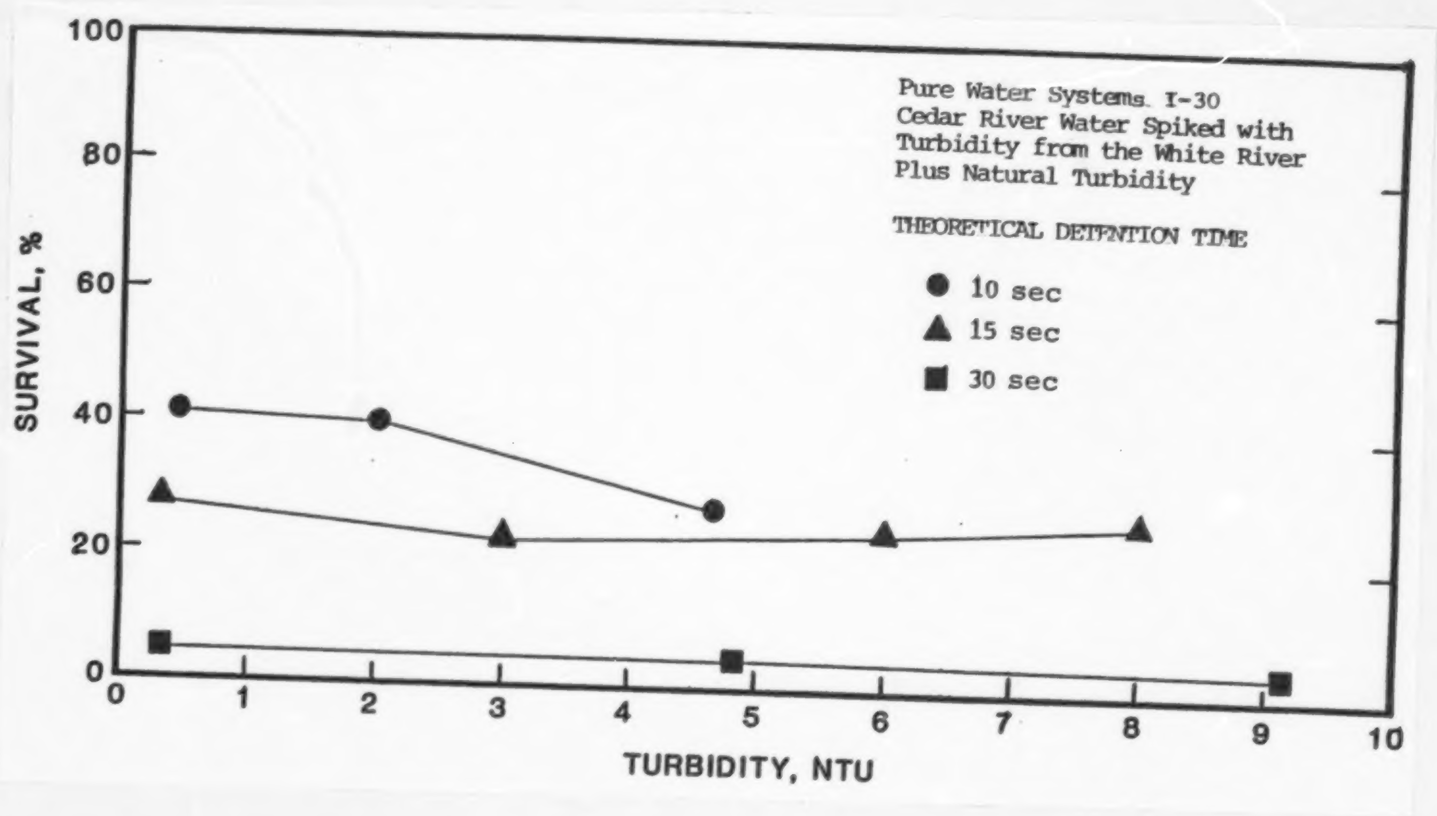


Figure 31. Effect of turbidity from White River on UV disinfection of *Giardia muris* cysts at various theoretical detention times for Pure Water Systems unit (with trapped gas).

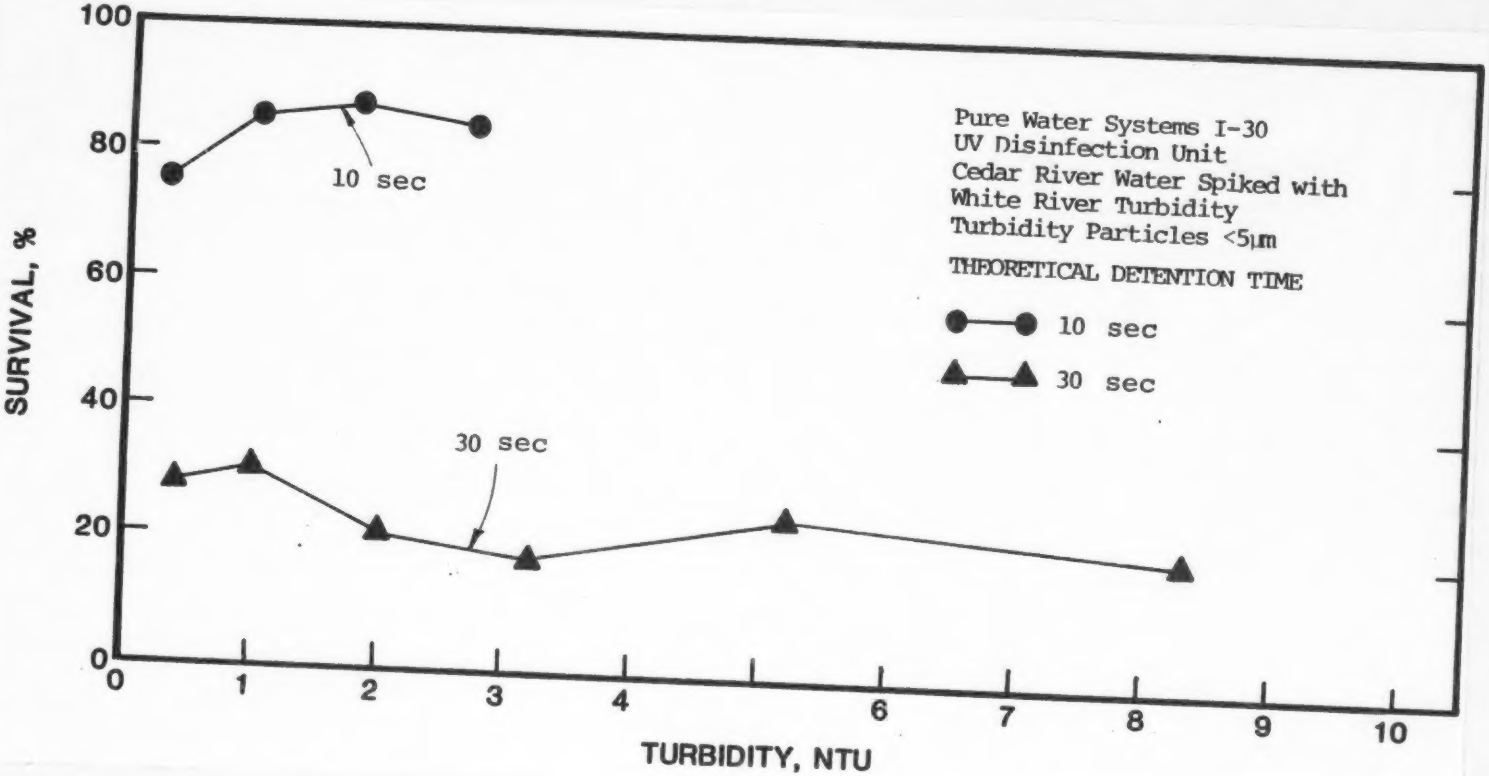


Figure 32. _Giardia_ _muris_ cyst survival vs. turbidity, White River through Pure Water Systems unit.

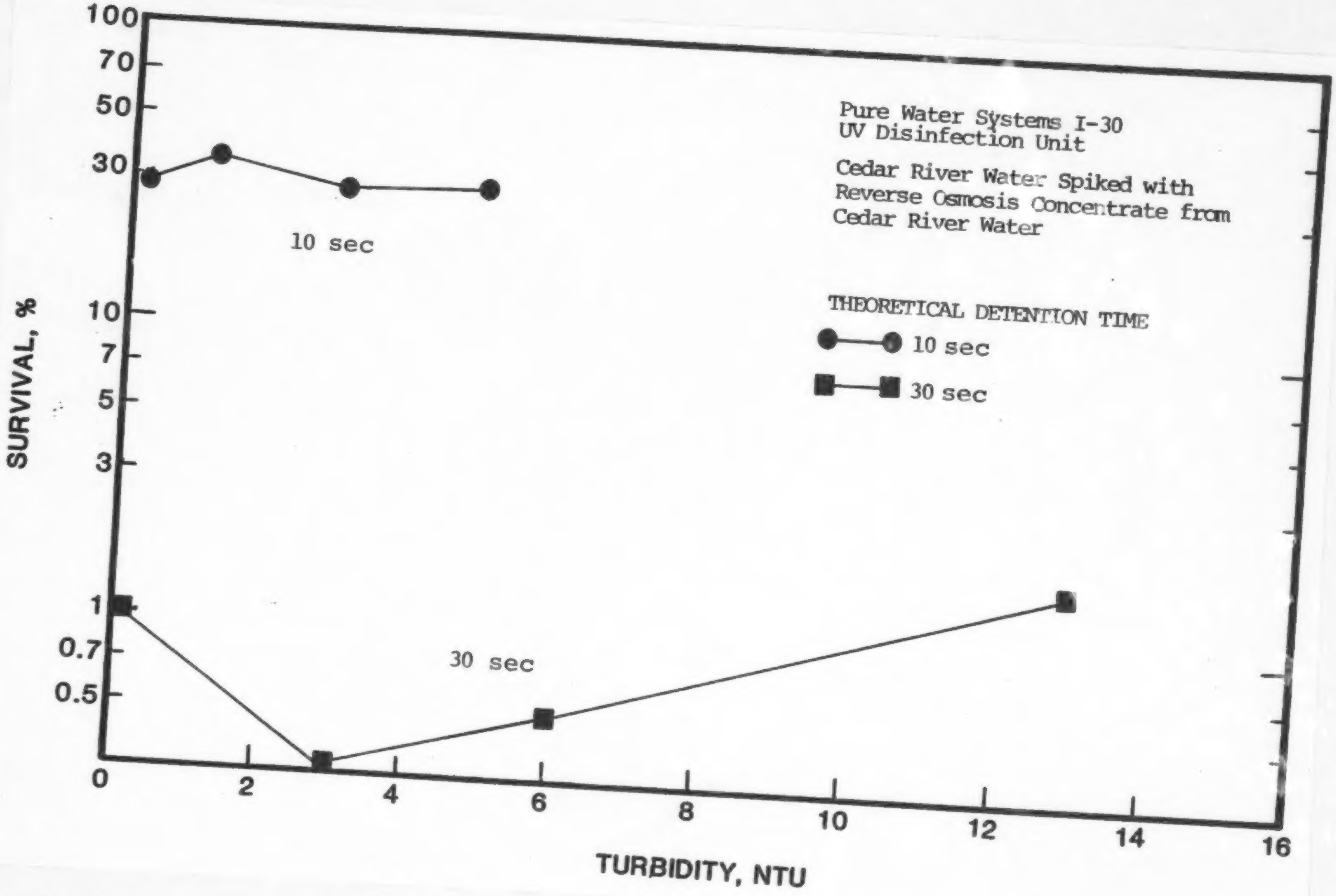


Figure 33. *Giardia muris* cyst survival vs. turbidity from reverse osmosis concentrate of Cedar River through Pure Water Systems UV unit.

TABLE 11. WATER QUALITY CHARACTERISTICS, WHITE RIVER TURBIDITY SPIKE

	White River Run 1	White River Run 2	ROCCR Run 1
pH	7.2-7.4	7.1-7.4	7.2-8.2
Temperature, oC	15.0-17.0	15.3-17.4	14.7-15.4
Conductivity, µmhos	46.0-62.0	45.0-51.0	52.0-66.0
D.O., mg/L	7.6-8.7	7.9-8.6	10.3-12.6
T.Alk., mg $CaCO_3$/L	22.0-22.6	21.8-25.0	22.2-36.0
T. Hardness,mg $CaCO_3$/L	23.6-24.8	24.2-28.3	23.4-34.6
Cyst Concentr., cyst/mL	254,327	170,271	231,249
Absorbance @ 254 nm, /cm	0.013-0.08	0.009-0.106	---

Evaluation of the turbidity-inactivation studies did not reveal any trend in the data. This is in contrast to the trends seen in the color-inactivation studies, which are easily explained by Beer's Law. The low number of data points and high degree of scatter in the data contributed to the difficulty in defining any trends.

Comparison between Figures 31 and 32 strikingly indicates the interference to UV disinfection of the cysts caused by the entrapped gas. Cyst survival at a retention time of 30 seconds in the presence of White River particulates dropped from an average of 23 percent to 4 percent after air was bled from the reactor. A similar increase in cyst inactivation was seen at 10 seconds where cyst survival decreased from 82 percent to 36 percent.

For the ROCCR runs, cyst inactivation was noticeably lower than for any previous runs for a given retention time using White River particulates. This difference was not due to the use of different sources of turbidity as this difference was also exhibited without a turbidity spike. An explanation which could result in increased intensity reaching the cysts was a difference in the transparency of the quartz tubes surrounding the UV lamps. During all runs, the quartz tubes were cleaned by means of the PWS's air powered wipers. Unfortunately, there was no way to determine just how well the tubes had been cleaned.

Considering that the difference between runs lies within the standard deviation for the excystation technique, the authors believe that the variability of the excystation technique was the probable cause for the resulting differences.

Several investigators have reported on the role particulates and/or turbidity play in the UV disinfection process. Qualls and Johnson (1983) found that filtered secondary wastewater irradiated with UV yielded greater coliform reductions than unfiltered secondary wastewater irradiated with UV. They concluded that the effect was primarily due to removal of large particles which were harboring or protecting bacteria. Although a relatively small number of coliforms were protected inside particles, these particles were the limiting factor for disinfection to -3 to -4 log survival. Similar reports of "particle-harbored bacteria" were presented by Roeber and Hoot (1975). Irradiated activated sludge effluents associated with high turbidity showed a significant increase in the coliform MPN when samples were blended after irradiation. This increase was not seen for blended samples from low turbidity effluents. Britton et al. (1972) observed that protection against UV inactivation was provided to Klebsiella aerogenes by various clay minerals. This protection was found to be dependent on the specific light absorption and scattering properties of the clay minerals. Concentrations of clay particles used in the study were in the range of 1000 to 10,000 mg/L. They found that irradiation of poliovirus in turbid seawater, as reported by Hill et al. (1967), was also adversely affected by turbidity.

In light of the references cited, there appears to be several reasons why this study did not witness any comparable adverse effects due to the presence of turbidity.

The greater percentage of particles used in the turbidity spikes were less than 5 μm in size because filtering was necessary in order to make the excystation slides readable. Comparing this to the physical dimensions of a Giardia cyst, 5 μm by 10 μm, it does not appear likely the particles could provide any protection by shielding or scattering, unless a great concentration of particles were present. Protection may be afforded if the particles could completely surround the cyst. However, given the short time for intermixing and the negative surface charge of both the cyst and particles, there was probably little interaction between the cyst and particles before irradiation. From these data, one can only speculate whether different results would have been seen if a different manner of particle to cyst mixing ratio had been used before irradiation, or a different particle size preparation had been used in the experiments.

The turbulent flow regime of the PWS-UV reactor probably also played an important part in reducing the interference effect of the particles. The Reynolds Number for the PWS-UV reactor was calculated to be approximately 2980, which is usually considered to be within the turbulent flow regime. The turbulent flow should significantly reduce any shielding that the particles could impose. The baffles within the reactor which provide helical flow, also contribute to disrupting any interference these particles may provide.

Ultraviolet Technology UV Unit--

A second commercially available UV disinfection reactor manufactured by Ultraviolet Technology, Inc. (UTI) was evaluated to compare its disinfection capability to that of the PWS-UV reactor. The evaluation of the UTI unit was based primarily on the inactivation of G. *muris* cysts suspended in Cedar River water. Cyst inactivation studies did not evaluate the effects of turbidity and color as was done for the PWS-UV reactor. General characteristics of the water quality during evaluation of the UTI unit are shown in Table 12.

TABLE 12. WATER QUALITY CHARACTERISTICS OF CEDAR RIVER WATER DURING ULTRAVIOLET TECHNOLOGY-UV EVALUATION

Run No.	Date	Absorbance (/cm)	pH	T (°C)	D.O. (mgO_2/L)	Cond. (µmhos/cm)	T.Alk.	T.Hard.
							($mgCaCO_3/L$)	
1	11/10/83	0.036	7.0	12.8	>12.0	32	19.4	20.2
2	3/8/84	0.044	7.0	13.1	>12.0	29	19.6	20.0
3	3/11/84	0.045	7.1	13.4	>12.0	32	20.4	21.0

Three experiment series were completed in the UTI evaluation. Results of the runs are presented in Figure 34 as percent G. *muris* cyst survival versus theoretical detention time. For detention times up to 10 seconds, cyst survival fell rapidly from 100 percent to 40 percent, after which the rate of inactivation slowed considerably, such that survival levels only dropped to approximately 20 percent for a detention time of 20 seconds.

The unexpectedly high survival levels in the UTI were similar to the results with the PWS when entrapped gas was a problem. As the intake for the pilot plant was below a spillway, and the temperature of the river water was considerably lower than the temperature of the laboratory trailer, sufficient opportunity existed for the release of gas from the water during flow through the pilot plant. However, the UTI constant-diameter Teflon reaction chamber was thought to relieve the UTI of problems with entrapped gas. However, upon examination, considerable pockets of gas were found within the reaction chamber. By increasing the flow rate for a flushing effect, elevation of the outlet of the UTI unit and firm tapping of the Teflon reaction chamber, most of the gas was expelled. For subsequent runs, i.e., the second and third runs, gas pockets were carefully purged from the reactor before each experiment.

In the second and third series of runs adequate purging of gas pockets increased the performance of the UTI considerably as shown in Figure 34. Whereas approximately 40 percent survival was obtained at a detention time of 10 seconds for the first run, the same detention time in the second and third runs reduced the cyst concentration to less than 10 percent of the original concentration. In fact, survival levels of one percent or less at detention times longer than 16 seconds were achievable. This is equivalent to a flow rate of 4.2 L/min (1.1 gpm), which is lower than the manufacturer's recommended flow rate, but not unrealistic for practical applications.

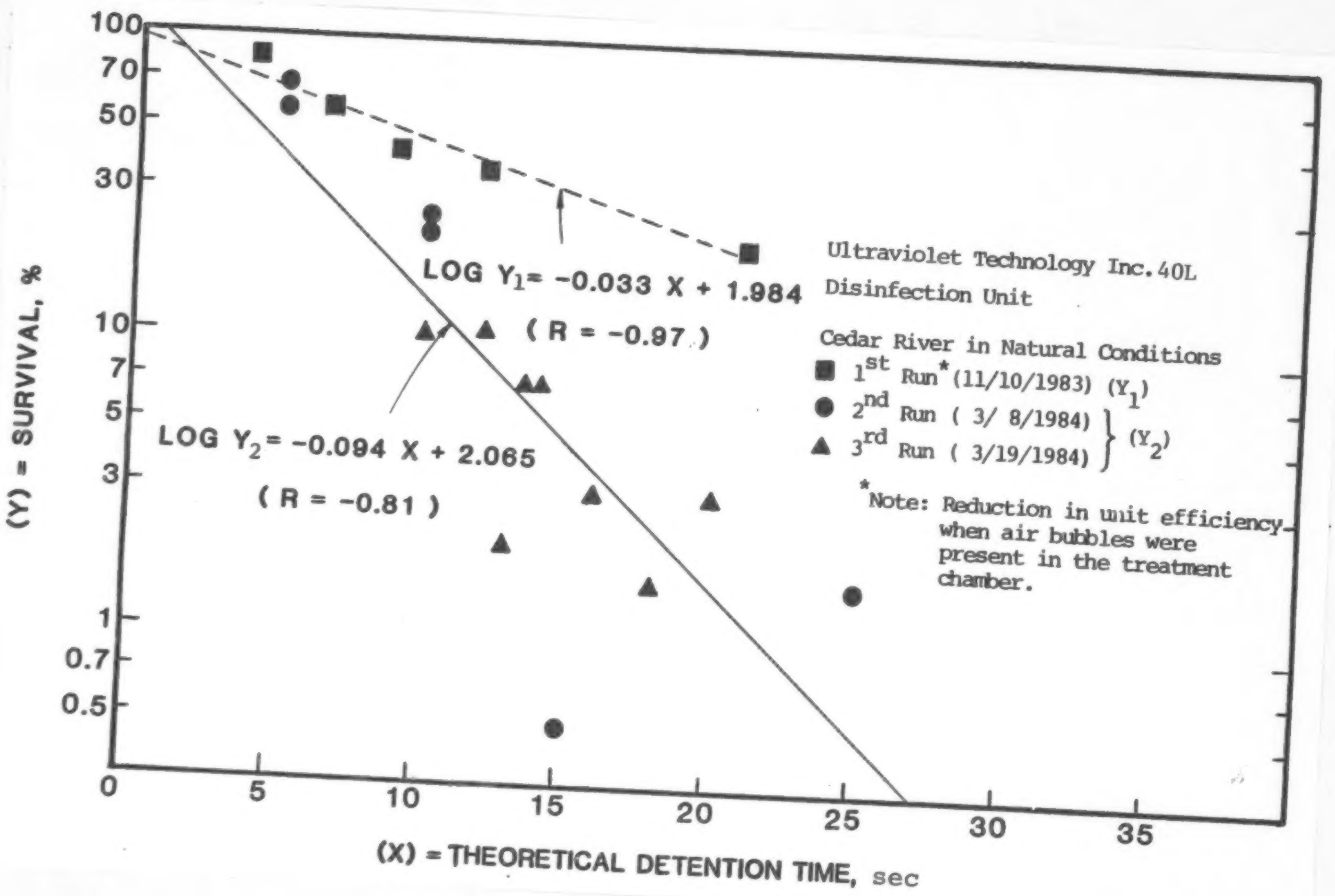


Figure 34. Survival of Giardia muris cysts vs. theoretical detention times for Ultraviolet Technology UV unit.

The ability of the UTI-UV reactor to attain a given level of inactivation at a shorter theoretical detention time than the PWS-UV reactor was a result of the higher intensity radiation reaching the cysts. For the PWS reactor, detention times longer than 60 seconds were required to achieve 1 percent survival. In contrast, detention times longer than about 22 seconds were required to reach the 1 percent survival level for the UTI unit.

However, if cyst inactivation is evaluated with respect to UV dose, Figure 35 shows that the UTI unit dosage is applied more efficiently than in the PWS. This is apparently due to the hydraulic flow profile of the reactor. As discussed previously the extent of short-circuiting in a reactor had a direct result on the level of disinfection obtainable, and the extent of short-circuiting through the PWS unit was considerably greater than through the UTI unit.

Laser-Generated UV--

Laser generated UV has a considerably greater intensity (mW/cm^2) than mercury-vapor fluorescent UV lamps. But as detention times for the laser pulse are on the order of 10 nanoseconds, equivalent dose ranges can be obtained for both sources. The high degree of coherence for the laser generated UV provided an excellent opportunity to study the effect of a narrow UV wavelength without the hydraulic complications of a vapor lamp reactor.

Laser inactivation studies consisted of three different suspension media: (1) distilled water, (2) turbidity suspensions, and (3) color suspensions. Both the turbidity and color suspensions were prepared from Cedar River reverse osmosis retentate (ROCCR). The G. muris cyst concentration for all exposures was 100,000 cysts/mL. Color and turbidity suspensions were diluted to various concentrations with distilled water.

A Giardia cyst suspension of 1 mL, placed in a 1 cm quartz cuvette, was exposed to laser-generated UV pulses. The cuvette was held no longer than 10 seconds in the laser path during exposures. Exposures were divided into 10 second segments in order to shake and mix the contents of the cuvette, thus reducing problems with settling and shielding. After exposures, Giardia cyst samples were excysted to determine percent survival of the cysts.

During preliminary work with the laser, the laser's pulse power was consistent and predictable. However, it deteriorated during the course of the study and the laser's power consistency during these reported Giardia inactivation tests was severely hindered. Therefore, the total energy delivered had to be back calculated from pulse readings measured through the cuvette-cyst suspension using transmittance factors which were determined when laser pulse power was predictable.

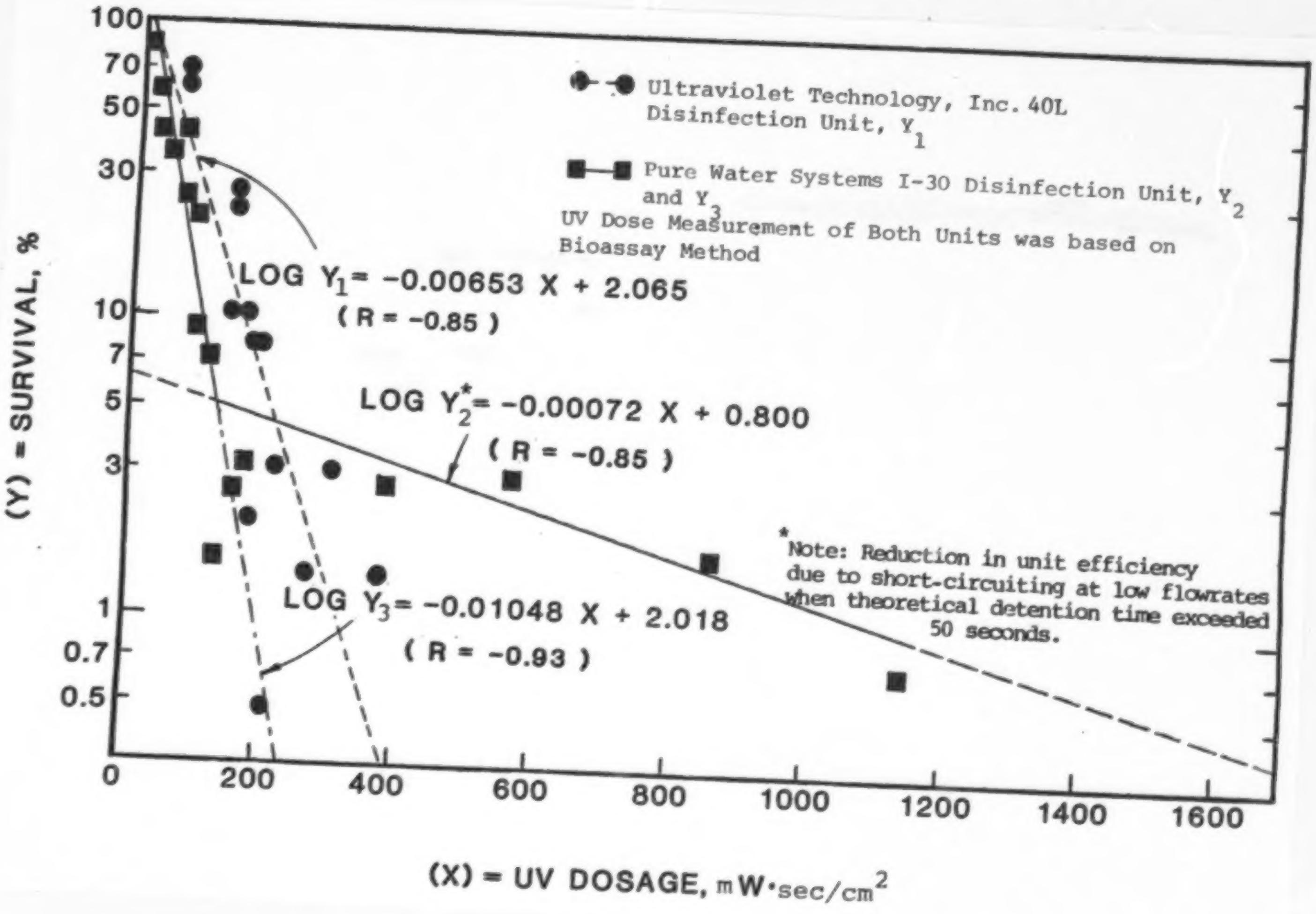


Figure 35. Percent survival vs. UV dose for Ultraviolet Technology and Pure Water Systems units.

Tables 13, 14, 15, 16, 17 and 18 present the experimental conditions and results for two series of exposures. Figure 36 shows the inactivation of cysts suspended in distilled water for both series of tests. The semilog plot of cyst survival (percentage) versus total UV dose delivered (mW-sec/cm^2) approximates a linear relationship. The line appearing in Figure 36 was obtained by linear regression analysis.

Comparison of the excimer laser-UV and PWS (mercury vapor lamp) effectiveness to inactivate *Giardia* cysts is shown in Figure 37. The curves as presented show a significant difference in the ability of the sources to inactivate cysts. However, the dosages used for the two curves were measured differently. Dosage indicated for the PWS is the *average dose received* across the cross-section of the contactor measured by bioassay methods while the dose of the excimer laser UV is the *energy delivered* measured by oscilloscope readings. With reference to Figure 37 the data suggested that the PWS unit was much more effective than the excimer laser unit in inactivation of *Giardia* cysts. The superiority of the PWS unit is more dramatic when the slope of the PWS curve ($\log Y_2 = -0.01048 X + 2.018$) is compared with the slope of the laser curve ($\log Y_1 = -0.00086 X + 1.720$).

UV Inactivation of Yersinia and Coliform Cultures

Figure 38 shows the UV inactivation curves (log survival vs. UV dose [exposure time]) for *Y. enterocolitica* 103 (virulent), *E. coli*-NAR (antibiotic resistant), and *E. coli* 25922 (non-antibiotic resistant). These curves were developed using the batch reactor and Pen Ray UV light source. Initial concentrations of the microorganisms were between 1×10^6 and 1×10^7 per mL. Data points represent the average value for four or five batch runs. *Yersinia* were enumerated on MacTween agar and *E. coli* were enumerated on EMB agar, with and without Nalidixic Acid.

All four organisms demonstrated a significant rate of inactivation in the first five minutes of irradiation (27 mW-sec/cm^2). In fact, the rate of inactivation for *E. coli* 25922 was great enough to attain "100%" inactivation in less than five minutes. As irradiation continued beyond five minutes, the inactivation rates for the remaining organisms began to slow to the extent that significantly long exposure times (high doses) would be required to attain the "100 percent" inactivation *E. coli* had attained.

Virulent *Yersinia* were the most resistant of the four organisms, both in regard to the initial rate of inactivation and the total log reduction attained. In contrast, although the initial rate of inactivation for *Y. enterocolitica* 158 and *E. coli*-NAR closely approximate the rate for *Y. enterocolitica* 103, total log

TABLE 13. SURVIVAL OF *GIARDIA MURIS* IN DISTILLED WATER SUBJECTED TO VARIOUS EXCIMER LASER UV DOSAGES

Carrier: Distilled Water		
Calculated Laser UV Dosage[1] mW-sec/cm^2	Measured Laser UV Residual[2] mW-sec/cm^2	% Survival of *G. muris* Cysts
117	86	47.0
321	236	47.9
583	430	21.3
773	570	18.8
1156	852	6.2
1749	1288	1.5
2487	1832	0.5
4711	3471	0.5

Laser Voltage=28 kV, preionizer @ 100%, pressure=2100 abs. torr
Cyst Concentration=100,000 per mL.

[1] The laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

[2] UV residual reaching the monitor after passing through the test water sample.

TABLE 14. SURVIVAL OF *GIARDIA MURIS* IN SEATTLE WATER WITH COLOR SUBJECTED TO VARIOUS LASER UV DOSAGES

Seattle water with color at various levels as measured by absorbance			
Absorbance Indicating Color Added cm^{-1}	Calculated Laser UV Dosage[1] mW-sec/cm^2	Measured Laser UV Residual[2] mW-sec/cm^2	% Survival of *G. muris* Cysts
0.00	583	430	21.3
0.06	612	386.5	20.5
0.12	630	365	23.6
0.19	773	386	29.5
0.25	1030	380	20.7
0.46	1298	376	20.7

Laser Voltage=28 kV, preionizer @ 100%, pressure=2100 abs. torr
Concentration=100,000 per mL.

[1] The laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

[2] UV residual reaching the monitor after passing through the test water sample.

TABLE 15. SURVIVAL OF GIARDIA MURIS IN SEATTLE WATER WITH ORGANIC TURBIDITY SUBJECTED TO VARIOUS EXCIMER LASER UV DOSAGES

Seattle water with organic turbidity added			
Organic Turbidity as Indicated by NTU	Calculated Laser UV Dosage[1] mW-sec/cm²	Measured Laser UV Residual[2] mW-sec/cm²	% Survival of G. muris Cysts
0	583	430	21.3
2.6	568.3	419	18.0
6.3	682.5	484	38.4
11.0	633.9	440	39.5
16.0	610.6	390	30.2
20.0	711.7	415	34.3
47	766.3	404	26.3

Laser Voltage=28 kV, preionizer @ 100%, pressure=2100 abs. torr
Cyst Concentration = 100,000 per mL.

[1] The laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

[2] UV residual reaching the monitor after passing through the test water sample.

TABLE 16. SURVIVAL OF GIARDIA MURIS IN DISTILLED WATER SUBJECTED TO VARIOUS EXCIMER LASER UV DOSAGES

Distilled Water	
Calculated Laser UV Dosage[1] mW-sec/cm²	% Survival of G. muris Cysts
170	21.0
194	29.0
333	34.0
369	24.7
660	21.0
702	10.7
729	6.5
1008	7.5
1025	8.1
1316	2.2
1681	1.0
2057	<0.7
2196	1.6
2686	1.0

Laser Voltage=28 kV, preionizer @ 80%, pressure=2150 abs. torr
Cyst Concentration=100,000 per mL

[1] The excimer laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

TABLE 17. SURVIVAL OF *GIARDIA MURIS* IN SEATTLE WATER WITH COLOR SUBJECTED TO VARIOUS EXCIMER LASER UV DOSAGES

Seattle water with color at various levels as measured by absorbance		
Absorbance Indicating Color Added cm^{-1}	Calculated Laser UV Dosage[1] mW-sec/cm^2	% Survival of *G. muris* Cysts
0.064	853	8.5
0.064	830	7.0
0.125	927	4.1
0.125	924	6.8
0.19	1092	11.4
0.19	1074	8.6
0.25	1496	7.1
0.25	1433	12.0
0.464	1805	5.3

Laser Voltage=28 kV, preionizer @ 80%, pressure=2150 abs. torr
Cyst Concentration=100,000 per mL

[1] The excimer laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

TABLE 18. SURVIVAL OF *GIARDIA MURIS* IN SEATTLE WATER WITH ORGANIC TURBIDITY SUBJECTED TO VARIOUS EXCIMER LASER UV DOSAGES

Seattle water with organic turbidity added		
Organic Turbidity as Indicated by NTU	Calculated Laser UV Dosage[1] mW-sec/cm^2	% Survival of *G. muris* Cysts
2.6	685	18.5
2.6	673	17.5
6.3	683	14.9
6.3	735	8.9
11.0	737	8.6
11.0	773	13.9
16.0	824	12.1
20.0	865	28.7
47.0	973	9.9

Laser Voltage=28 kV, preionizer @ 80%, pressure=2150 abs. torr
Cyst Concentration=100,000 per mL

[1] The excimer laser output of UV intensity was determined by measuring the UV that penetrated the sample to reach a monitor whose readout was on an oscilliscope, and then back calculating to determine the dosage entering the sample tested.

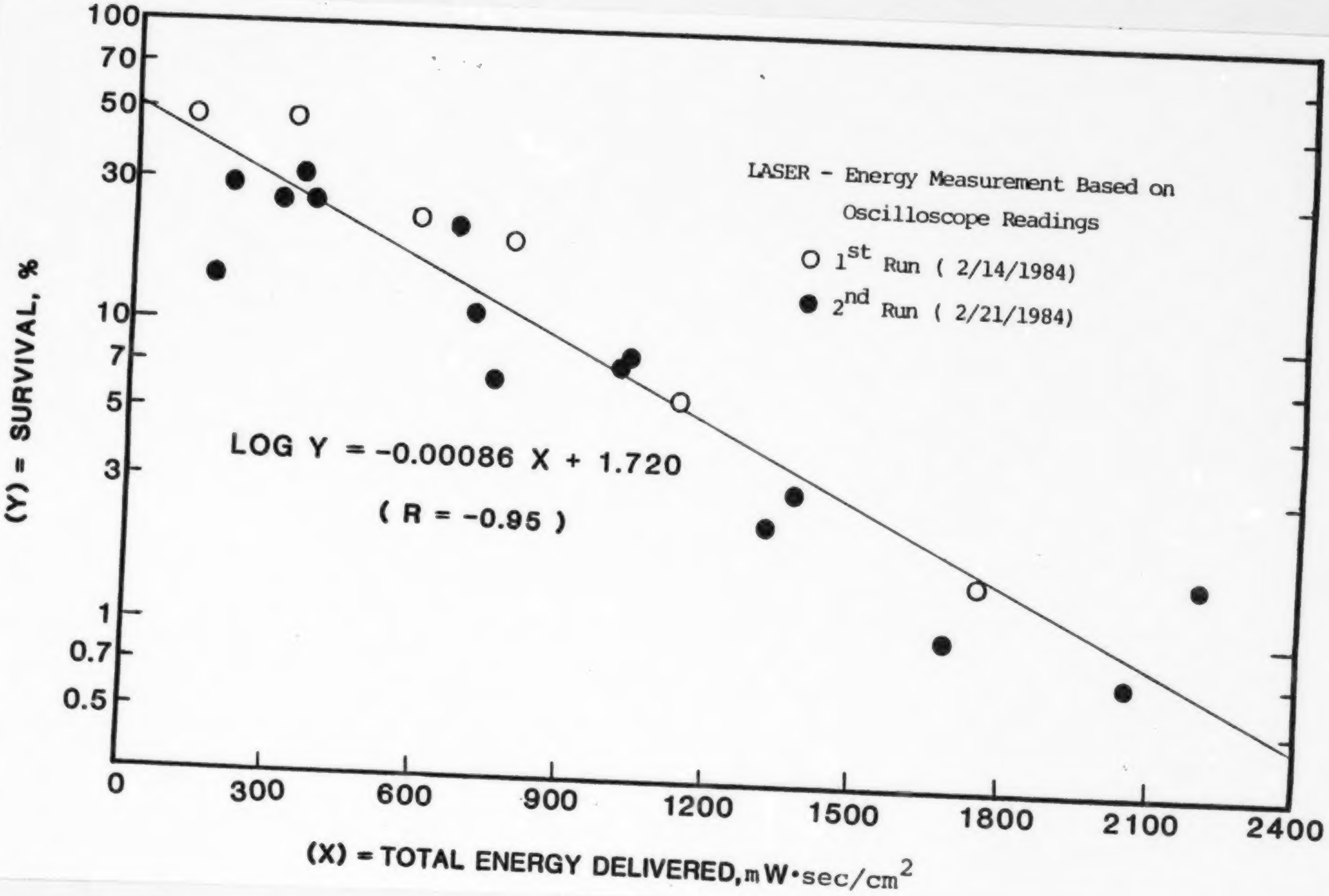


Figure 36. Giardia muris cyst survival vs. total energy delivered for laser generated UV.

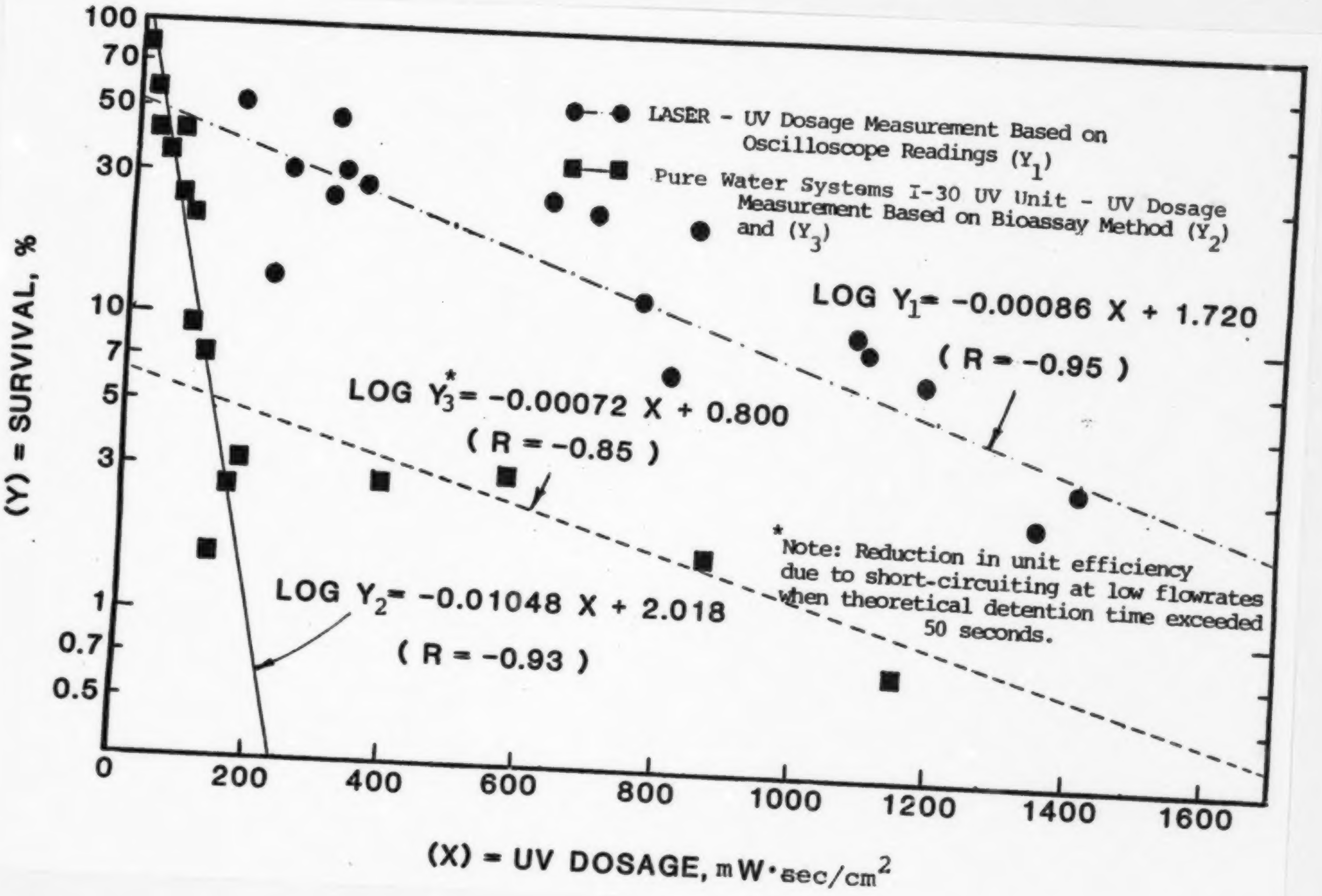


Figure 37. Giardia muris cyst survival vs. UV dosage for Pure Water Systems UV unit and laser generated UV.

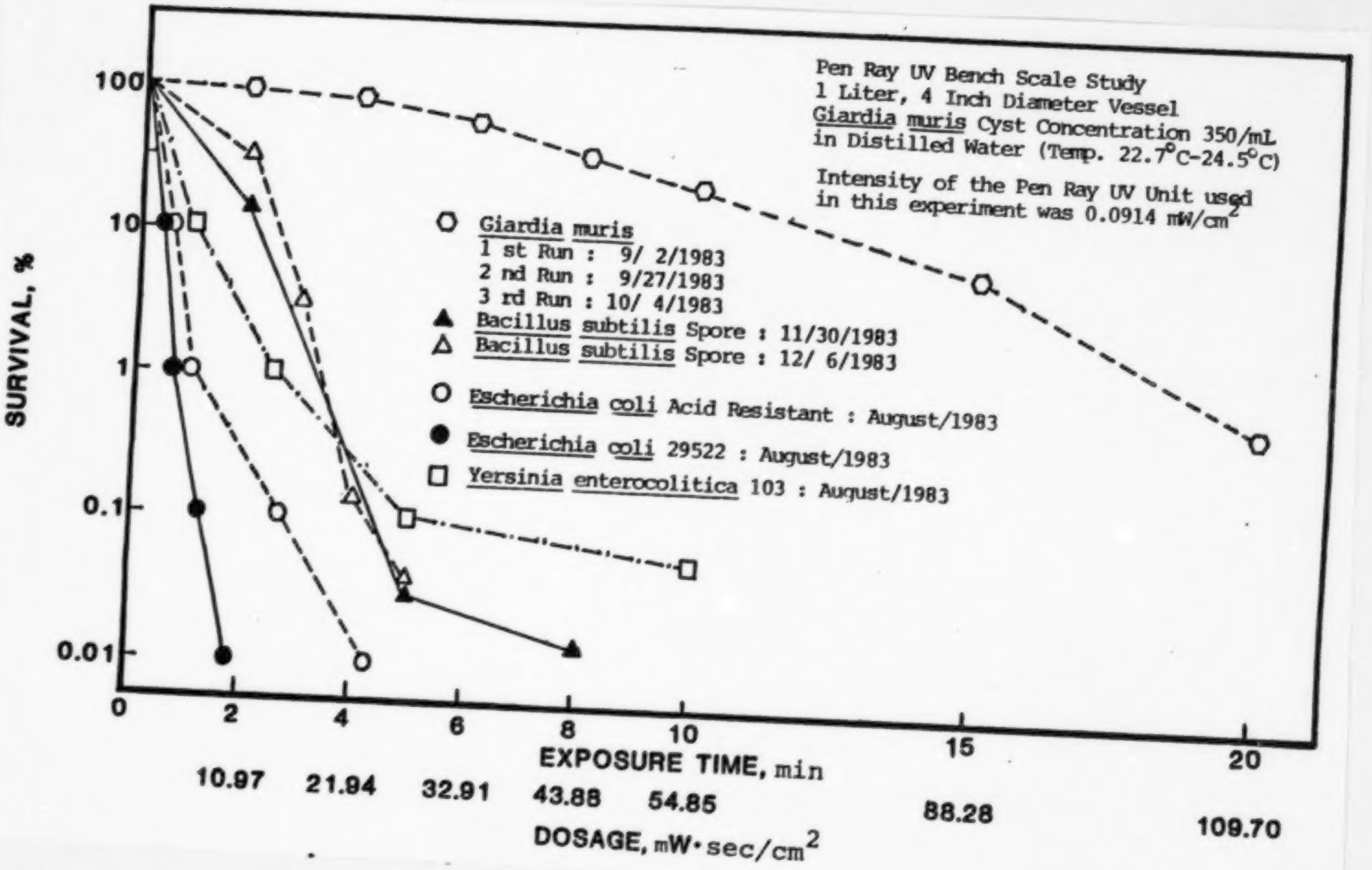


Figure 38. Comparison of Giardia muris survival vs. total UV dosage.

reductions for *Y. enterocolitica* 158 and *E. coli*-NAR were considerably greater: 6.5 and 5.5 log reductions, respectively. The concentration of survivors could be referred to as a "resistant" subpopulation. However, such a designation should be used cautiously because it is not known if these survivors are truly resistant to UV or merely products of a dynamic process.

The differences in susceptibility to UV disinfection between strains of the *Yersinia* and *E. coli* were of special interest. Virulent *Yersinia* (103), which contained a plasmid, demonstrated significantly greater resistance to inactivation by UV than did the non-plasmid containing counterpart - *Y. enterocolitica* 158. The suggestion that protection was associated with the plasmid was evaluated by curing virulent *Yersinia* of its plasmid and irradiating it in the batch UV reactor. The "cured" *Y. enterocolitica* did exhibit an inactivation curve quite similar to the curve for *Y. enterocolitica* 158, the non-plasmid *Yersinia* strain. This result further implicated the relationship between UV protection and the presence of plasmids.

Similarly, nalidixic acid resistant *E. coli*-NAR exhibited a greater resistance to UV inactivation than the non-NAR strain of *E. coli*. Unfortunately, there is no known way to remove the resistance to nalidixic acid from *E. coli*-NAR to determine whether the organism's antibiotic resistance could more directly be linked to the difference in UV sensitivity between the two strains. Antibiotic resistance and UV sensitivity have been addressed by Meckes (1982). He reported that UV inactivation of secondary wastewater effluent preferentially selected for the survival of coliforms resistant to the antibiotics tetracycline and chloramphenical. Although this finding was attributed to the mechanism of R-factor mediated resistance, no association was found between R-factors which might mediate resistance to UV and R-factors which mediate specific antibiotic resistance.

Comparison of UV inactivation curves for *Y. enterocolitica*, *E. coli*, and *G. muris* cysts graphically exhibits the tremendous resistance cysts have to UV inactivation. The fact that both *Yersinia* and *Giardia* cysts are more resistant to UV than *E. coli* has important implications where the total coliform procedure is used to monitor disinfection efficiency and indicate microbiological water quality. Outbreaks of giardiasis in water supply systems which reported satisfactory total coliform concentrations are indicative of the problems associated with using coliforms as indicator organisms for adequate UV disinfection.

PWS-UV Reactor Study

Three inactivation runs were performed to assess the PWS-UV reactor's ability to disinfect *Y. enterocolitica* and *E. coli*. Runs consisted of inactivation in unaltered Cedar River, Cedar River spiked with turbidity (ROCCR) and Cedar River spiked with color (ROCCR). *E. coli* were enumerated on different media for each run while only two different media were used to enumerate *Y. enterocolitica*. All runs evaluated a detention time of 15 seconds; only one run evaluated a detention time of 30 seconds.

Results from the *Yersinia*/*E. coli* inactivation runs were somewhat disappointing, both in regard to levels of inactivation and problems associated with media and background interference. Of the three runs, only the last two at a detention time of 15 seconds gave results which were consistent and without media problems. Significant problems encountered with background interference were the major reason various media were used.

For the first run, there was no difference between the level of inactivation of *E. coli* and *Y. enterocolitica* regardless of the quality of water. For all conditions under which testing was completed -- irradiation produced a log reduction of 1.99 or an approximate 99 percent inactivation. Unirradiated concentrations were 600,000/mL and 500,000/mL for *E. coli* and *Y. enterocolitica*, respectively. Low turbidity, low color, low hardness and alkalinity, and neutral pH characterized the Cedar River quality (see Table 12). Spiked runs obtained a turbidity of 8.5 NTU (background turbidity was 0.2 NTU) and the color spike raised the water absorbance from 0.013/cm to 0.18/cm. Table 19 presents the results of the run.

TABLE 19. INACTIVATION OF *YERSINIA ENTEROCOLITICA* AND *ESCHERICHIA COLI* IRRADIATED BY THE PURE WATER SYSTEMS UV REACTOR AT 165 mL/sec (15 sec DETENTION TIME)

Run 1

Water Condition	Inactivation			
	E. coli		*Y. enterocolitica*	
	log	%	log	%
Natural	1.998	99.0	1.999	99.0
Turbidity Spike	1.996	99.0	1.997	99.0
Color Spike	1.998	99.0	1.995	99.0

Results from the first run were plagued with problems related to enumerating the microorganisms. Some of the problems were associated with getting organisms to grow, particularly *Yersinia*. The other part of the problem was created by high background counts which made colony enumeration difficult.

The second run was set up to reproduce the conditions of the first run, however, different media were used to enumerate *E. coli* and an additional 30 second detention time was evaluated. For the 15 second detention time, results were more representative of results from the batch test. Turbidity values were raised from 0.3 NTU to 5.5 NTU with the turbidity spike, and the color spike raised absorbance from 0.017/cm to 0.27/cm.

Results from the second run are presented in Table 20. *E. coli* were inactivated to a greater extent than *Y. enterocolitica*, at the 15 second detention time, for all conditions except the turbidity spiked run. For natural conditions, log inactivation of *E. coli* and *Y. enterocolitica* were 2.11 and 1.78, respectively. When the color spike was added, inactivation values decreased significantly for both microorganisms. Log *E. coli* inactivation decreased to 0.51 and log inactivation dropped to 0.30 for *Y. enterocolitica*. The turbidity spike did not appear to have any significant effect on disinfection results except for *E. coli*. Inactivation decreased for *E. coli* while slightly more *Y. enterocolitica* were inactivated when turbidity was spiked into the Cedar River with the 15 second detention time.

TABLE 20. INACTIVATION OF *YERSINIA ENTEROCOLITICA* AND *ESCHERICHIA COLI* IRRADIATED BY PURE WATER SYSTEMS UV REACTOR AT 165 mL/sec AND 80 mL/sec.

Run 2

Water Conditions	Inactivation							
	165 mL/sec(15 sec detention time)				80 mL/sec(30 sec detention time)			
	E. coli		*Y. enterocolitica*		*E. coli*		*Y. enterocolitica*	
	Log	%	Log	%	Log	%	Log	%
Natural	2.11	99.2	1.78	98.3	2.08	99.2	3.4	99.96
Turbidity Spike	1.81	84.5	2.08	99.2	1.96	98.9	2.1	99.2
Color Spike	0.51	69.1	0.30	49.9	1.96	98.9	1.8	98.4

Unfortunately, data for the 30 second detention time appeared to be less reliable than the data from the 15 second detention time. Turbidity values reached 6.3 NTU during the spiked run, however, the color spike only brought water absorbance up to 0.15/cm. Greater inactivation of *Yersinia* than *E. coli* was obtained for natural Cedar River conditions. Turbidity and color spikes had little effect on the inactivation of *E. coli*. Log inactivation of *E. coli* only decreased from 2.08 to 1.96 for the spiked runs. In contrast, log inactivation values for *Yersinia* were considerably different when the Cedar River was spiked with either turbidity or color. The color spike decreased inactivation of *Y. enterocolitica* from 99.96 to 98.4% and the turbidity spike decreased inactivation from 99.96 to 99.2%.

The problems experienced with the 30 second detention time were probably the result of the low concentration of microorganisms spiked into the Cedar River water. Concentrations of *Y. enterocolitica* and *E. coli* in unirradiated samples were only 2500/mL. Much higher concentrations were expected and therefore dilutions for enumeration were much greater than required. Whether these low concentrations had an effect on inactivation was not determined. However, it would be expected that the probability of attaining a "hit" at lower concentrations of microorganisms would be smaller than for large concentrations.

Like the second run, the third run attempted to reproduce the conditions of the first run with only a 15 second detention time evaluated. *E. coli* were identified and enumerated on Tergitol-7 TTC and *Y. enterocolitica* were cultured on Tergitol-7. Turbidity spikes raised the natural turbidity from 0.2 NTU to 6.5 NTU. Absorbance was increased to 0.16/cm at 254 nm with the color spike. Unirradiated concentrations of *Y. enterocolitica* and *E. coli* were 560,000/mL and 650,000/mL, respectively.

Results from the third run are reported in Table 21. Survival concentrations were representative of the relative resistance the microorganisms exhibited in the batch study. Log inactivation of *E. coli* was 2.07 for natural conditions. This compares with the values of 1.988 and 2.11 attained for the first and second runs, respectively, under natural conditions. *Y. enterocolitica* had a much smaller level of inactivation for the third run than for any of the other runs. This is believed to be due to the use of the Tergitol-7 which give *Y. enterocolitica* colonies a very distinctive color. A slight increase in survival of both organisms was seen in the run made with the turbidity spike and a greater increase in survival resulted from the introduction of the color spike. These results are somewhat similar to Run 2 made at a 15 second detention time.

TABLE 21. INACTIVATION *YERSINIA ENTEROCOLITICA* AND *ESCHERICHIA COLI* IRRADIATED BY THE PURE WATER SYSTEMS UV REACTOR AT 165 mL/sec (15 sec DETENTION TIME)

Run 3

Water Condition	Inactivation			
	E. coli		*Y. enterocolitica*	
	Log	%	Log	%
Natural	2.07	99.1	1.07	91.5
Turbidity Spike	2.00	99.0	0.89	87.1
Color Spike	1.83	98.5	0.71	80.5

With reference to the results from the third run and the second run at a detention time of 15 seconds, increased absorbance correspondingly increases the level of survivors, which is simply explained by Beer's Law. On the other hand, turbidity did not affect UV inactivation of *E. coli* but slightly interferred with inactivation of *Y. enterocolitica*. Though survival concentrations were different for natural and turbidity spiked runs, these differences probably fall within the range of experimental error.

Levels of inactivation were much smaller than expected. Based on results of the *Giardia*:PWS runs and results from UV batch studies, a 15 second detention time through the PWS-UV reactor should have been able to attain a greater reduction in Yersinia than just one log. Using the simple hydraulic flow model, log reductions of at least 4 were expected, assuming the average light intensity in the reactor is 2.4 mW/cm^2. Similarly for E. coli, levels of inactivation were much smaller than expected. These low levels of inactivation are believed to be a result of size and morphological characteristics of the cells in comparison to suspended particulates. For example, shielding of these relatively small organisms may be significant in contrast to that of the larger Giardia cysts.

SECTION 6

ECOLOGICAL ASPECTS OF GIARDIA CYSTS

EFFECT OF TEMPERATURE ON GIARDIA SURVIVAL

A study on the effect of storage time on *G. muris* cysts at various temperatures was conducted. The 1°C, 5°C, 10°C and 20°C experiments showed differences in die-off rates as illustrated in Figures 39 and 40a and b. No excystable cysts were observed at 1°C, 5°C, 10°C and 20°C after storage periods of 120, 95, 63 and 26 days, respectively. The log of K' (decay coefficient) is plotted versus the inverse of temperature in Figure 40b. Figure 41, derived using the data from the previous figure, shows iso-percent survival curve for various storage times and temperatures.

SETTLING OF GIARDIA CYSTS IN QUIESCENT CONDITIONS

A well-insulated settling column of 15.3 cm (6 in.) diameter and 152.4 cm (5 ft) tall was used to determine the settling characteristic of *Giardia* cysts in quiescent water. The samples at different depths were collected periodically and analyzed for cyst concentration using a Coulter Counter. Figure 42 demonstrates percent cyst removal at each depth with time, for example, after 2 days approximately 50 percent of cysts were removed from the water column.

ZETA POTENTIAL

Zeta potentials (ZP) reported here are for *G. lamblia*. The measurements were carried out over a wide range of pH values, from a pH of 2 to just over 9. This was necessary in order to cover the pH of most natural waters and to pinpoint the cyst's isoelectric point. The test was repeated four different times, at 18, 48, 78 and 108 days, during which time the fixed and viable cyst suspensions were stored at 5°C. It can be argued that calling the cysts in the untreated suspension viable may be deceptive. The number of virulent organisms decreased steadily with each additional day of storage and most likely very few, if any, would be viable at the end of the experiment. However, because the cysts were not deliberately inactivated, a potential for finding virulent organisms would exist.

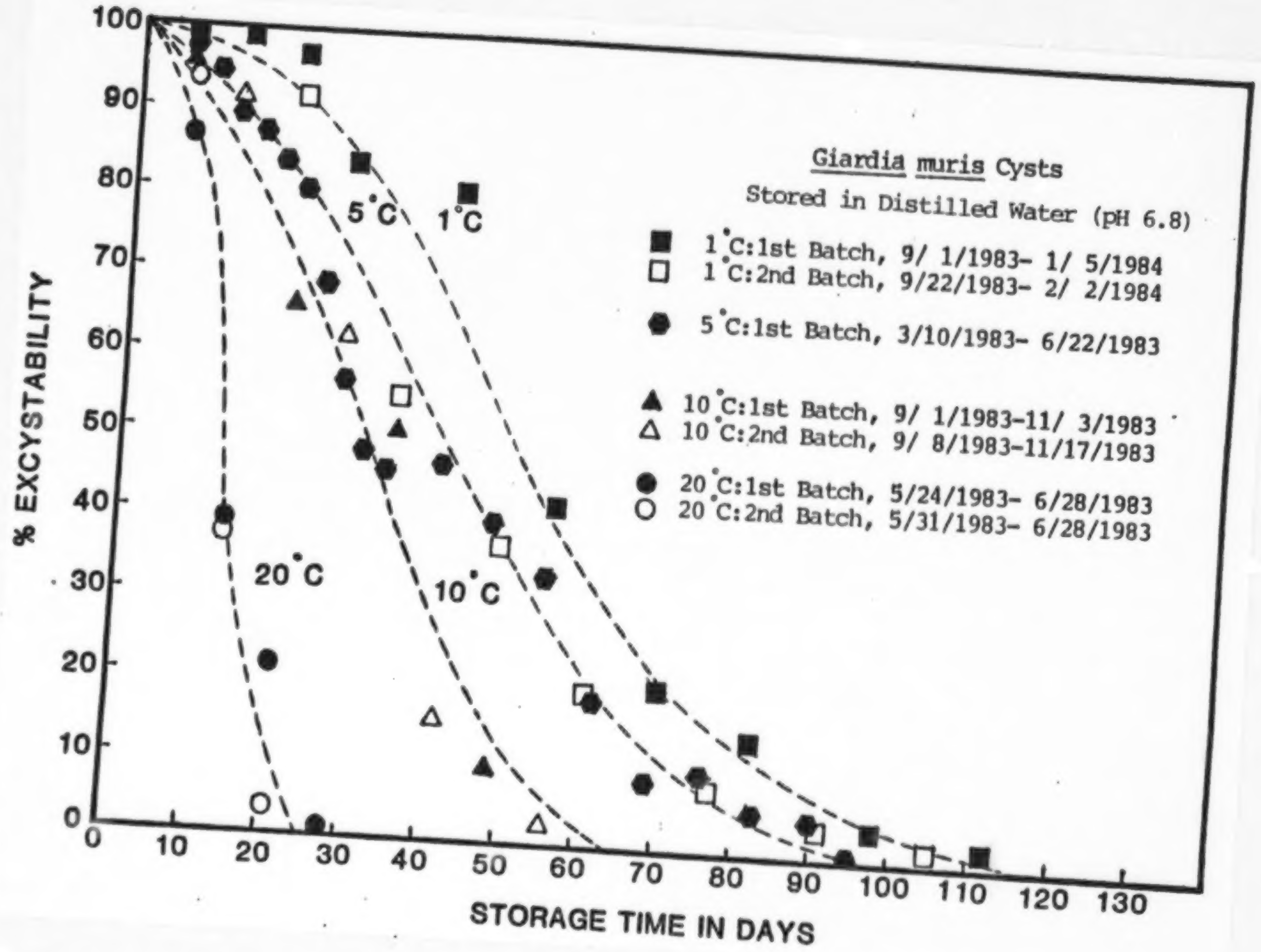


Figure 39. Giardia muris survivability at different temperatures.

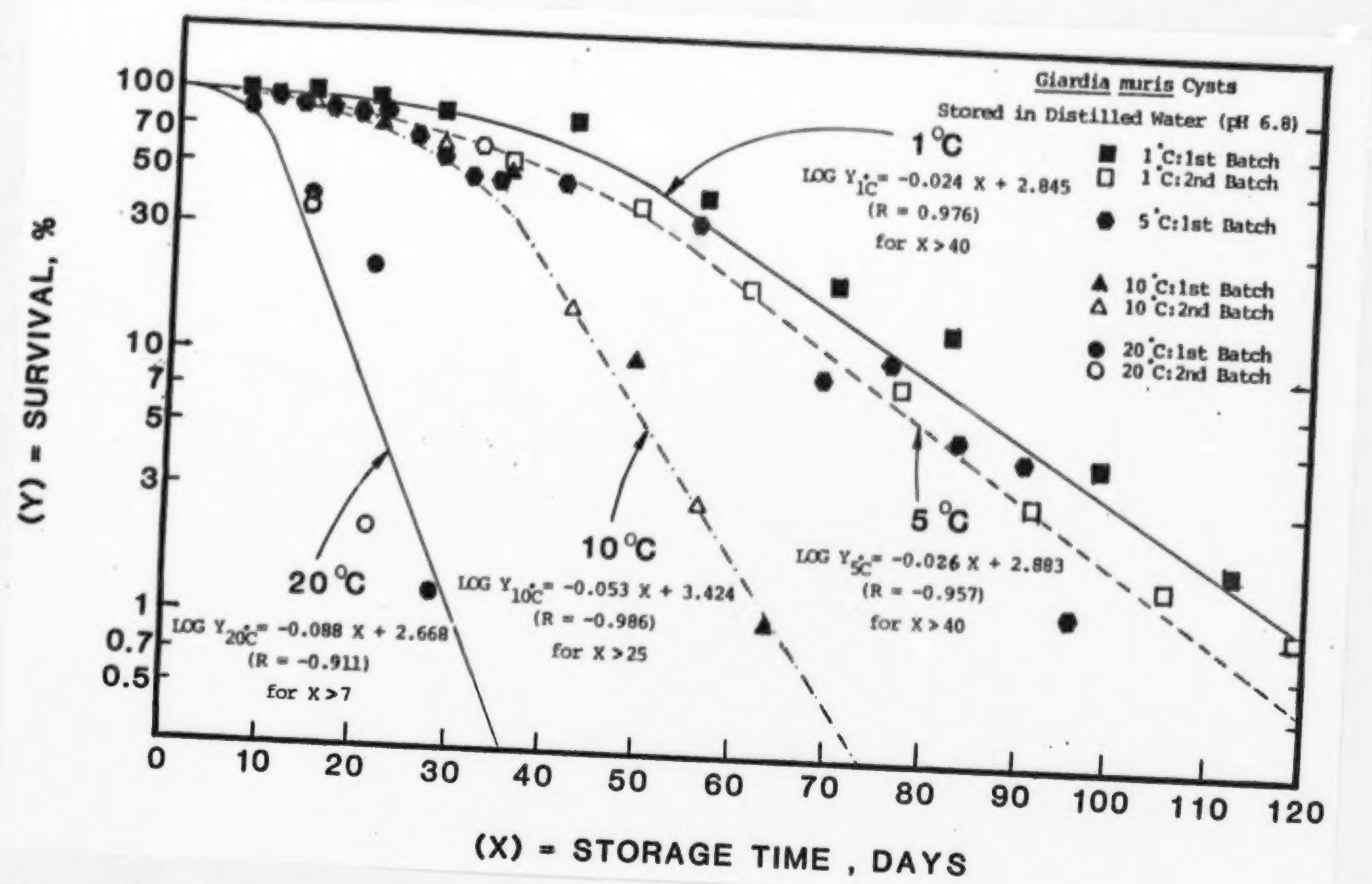
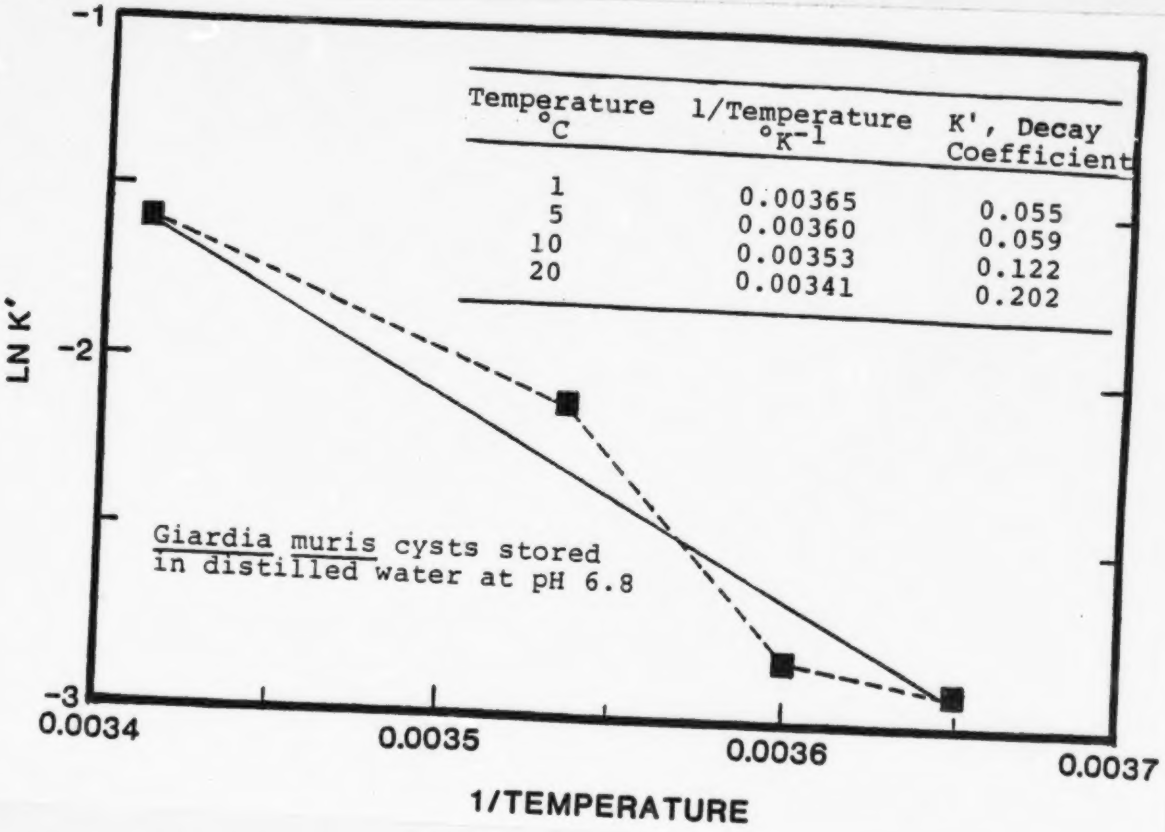


Figure 40a. *Giardia muris* cyst die-off curves due to storage at different temperatures.



Temperature °C	1/Temperature °K-1	K', Decay Coefficient
1	0.00365	0.055
5	0.00360	0.059
10	0.00353	0.122
20	0.00341	0.202

°K = degrees Kelvin

Figure 40b. Decay coefficient for viability of stored Giardia muris cysts at various temperatures.

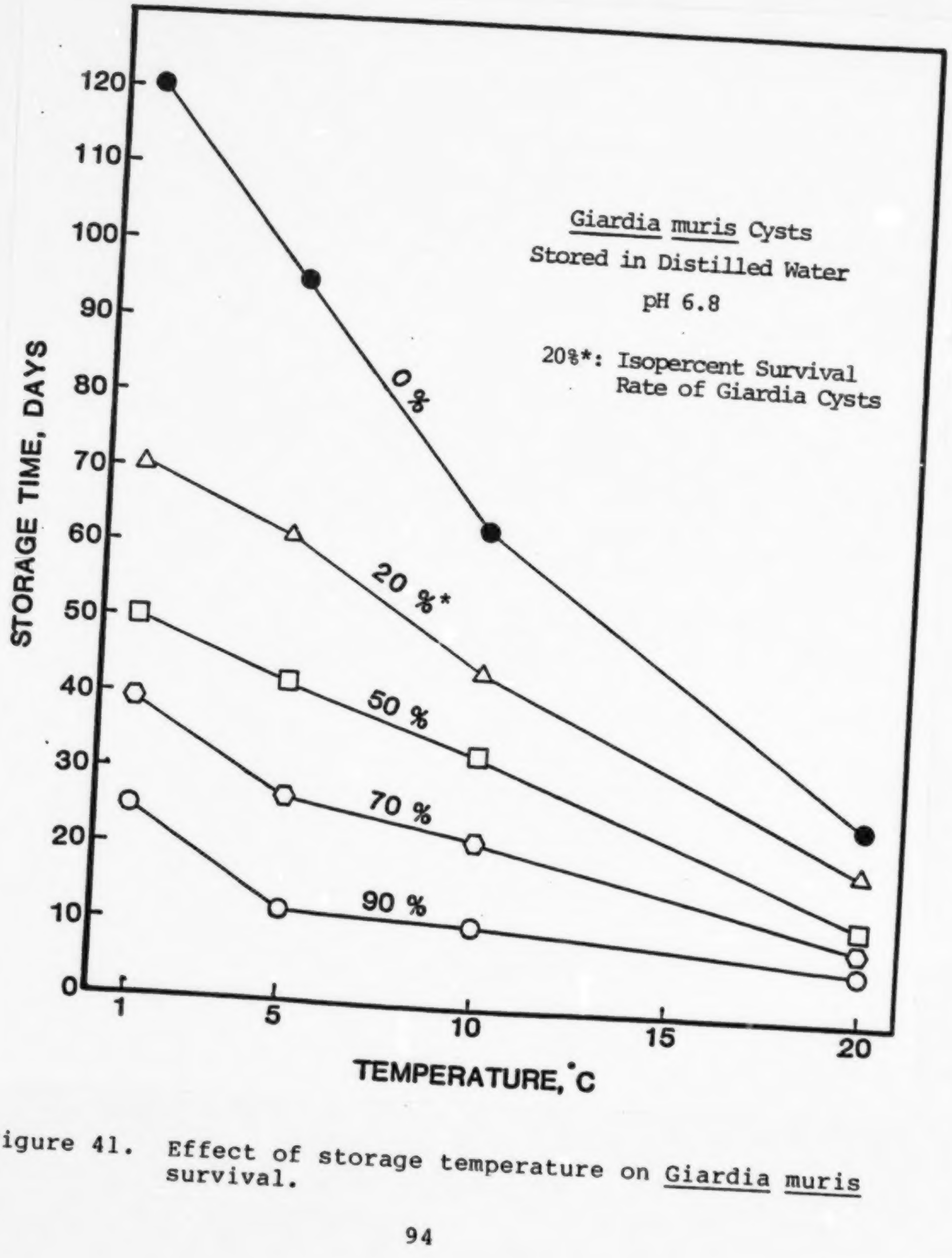


Figure 41. Effect of storage temperature on _Giardia_ _muris_ survival.

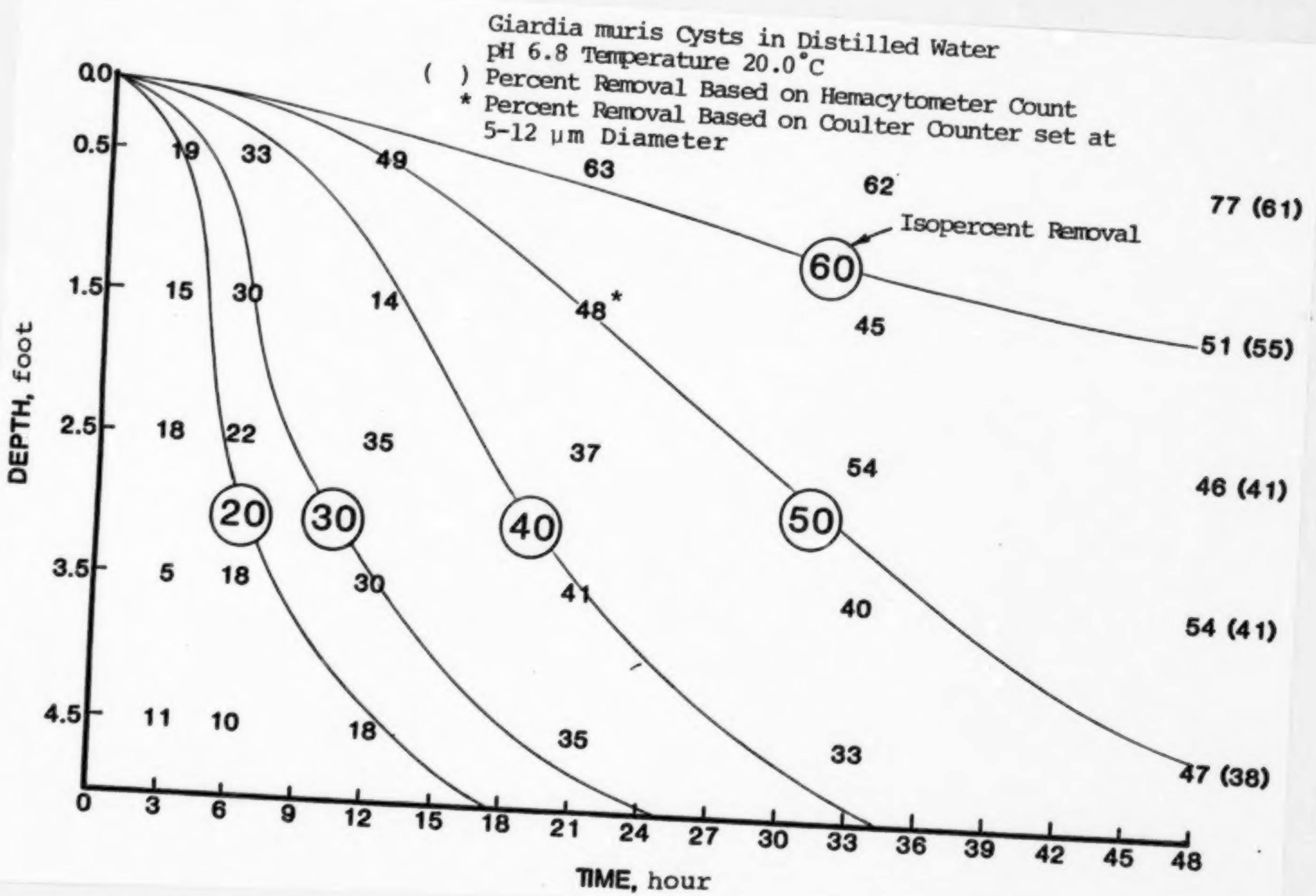


Figure 42. Settling paths of Giardia cysts as measured in the test cylinder using Coulter counter.

The variations in ZP for viable and fixed cysts as a function of pH are shown in Figures 43 and 44, respectively. Although the two sets of curves were quite similar, there were some differences. For the pH range of most natural waters, the formalin fixed cysts, in all four age categories, were found to be slightly more negative than the viable. The fact that the electric potential of the two groups was dissimilar was not surprising. Formalin is known to react readily with compounds containing an active hydrogen atom such as amino, imino, and peptide groups to form additive compounds such as hydroxymethyl. The additive compounds in turn react with other groups containing an active hydrogen atom which results in the formation of methylene bridges. The exact composition of the cyst wall is not known but conceivably one or more of these groups can be present. The resulting reactions with formalin would then change the chemical composition of the cyst surface and hence its electric potential.

During the three month testing period the ZP of the cysts showed a continual decline. From day 18 to day 108 the decrease was approximately 22 mV for both the viable and fixed cysts. At pH 7, that meant for the viable cysts an initial electric potential of -57 mV and a final of -35 mV. For the fixed cysts, the values were -63 and -41 mV, respectively.

Previous research by Logsdon *et al*. (1981) revealed that *G. muris* cysts in distilled water had a ZP of -26 mV at pH 5.5. No explanation is offered for these different ZPs for *G. muris* and *G. lamblia*.

The pH of the isoelectric point appeared initially to drop slightly with aging for both cyst groups, only to increase again for the final reading. However, the variations were minimal, from pH 3.1 to 3.5.

EFFECTS OF PRESSURE AND ALUM ON CYST STABILITY

During operation of the pilot plant it appeared that a large number of the cysts added to the plant's influent line were lost before reaching the filters. The losses were found to be on the order of 50 to 80 percent with alum coagulation when considering only the number of cysts added and recaptured. In an effort to determine the cause of this phenomenon, a series of tests were initiated.

First, an extensive reevaluation of the membrane filter recovery technique was made which yielded 80 to 90 percent capture of the cysts, averaging 85.6 percent as established earlier.

The next phase included an attempt to recover cysts from deposits on pipe walls, flocculator paddles, walls of the flocculator tanks, and other surfaces of the plant in contact with

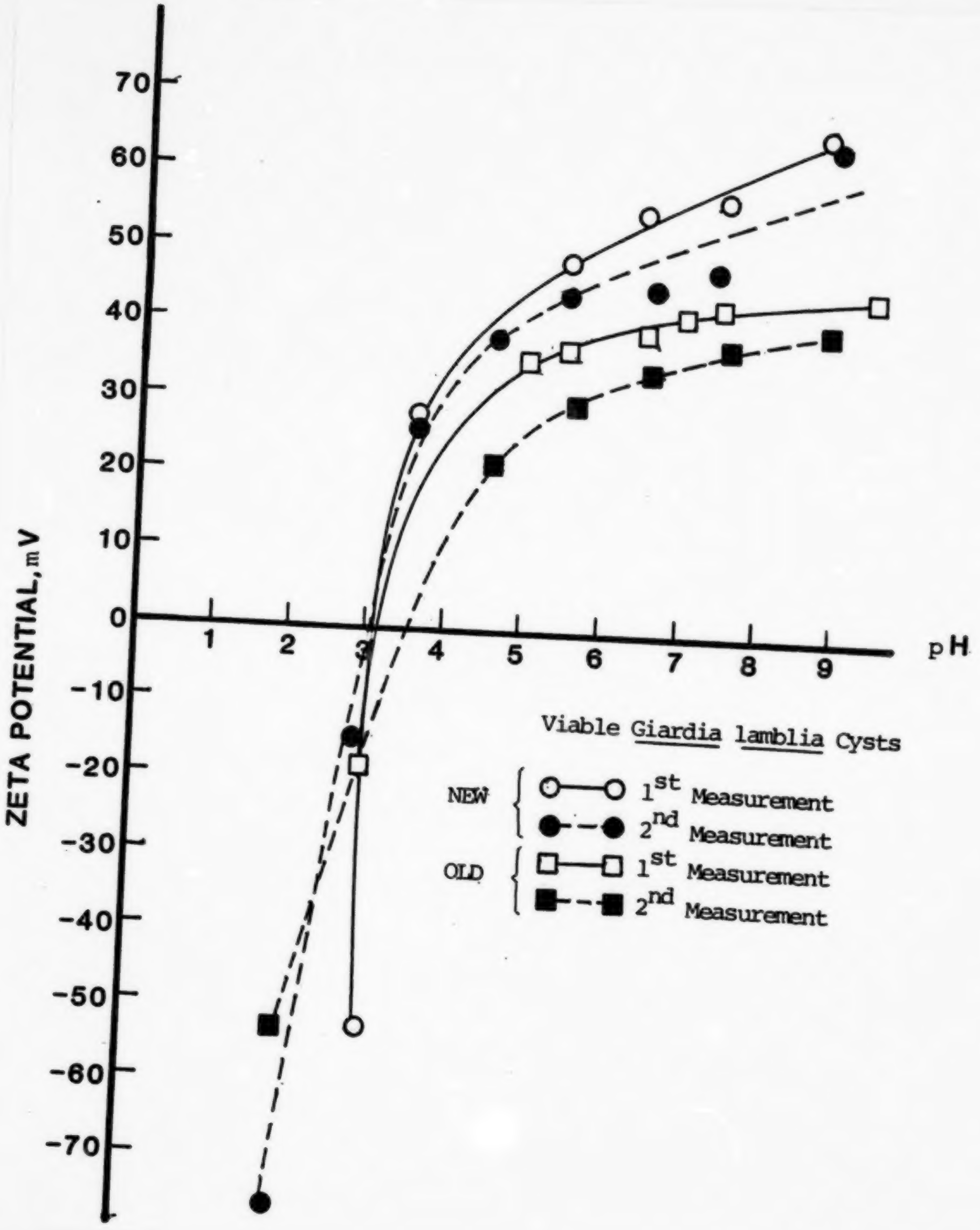


Figure 43. Zeta potential for viable Giardia cysts.

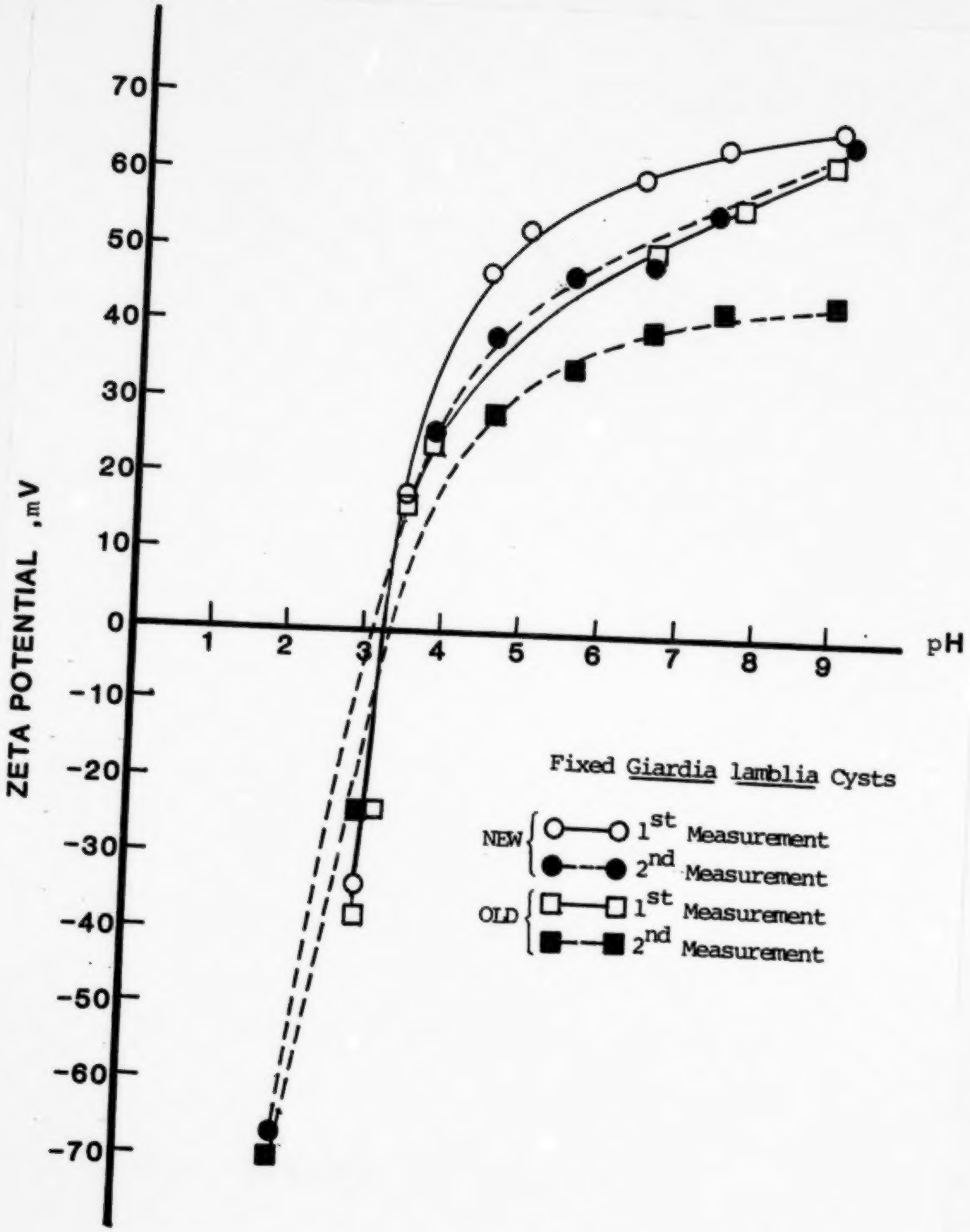


Figure 44. Zeta potential for fixed Giardia cysts.

the Giardia contaminated water. No cysts were obtained from any of these surfaces.

The pump used to feed the concentrated cyst suspension into the raw water line did not damage the organisms, nor did the mere exposure to alum concentrations of up to 25 mg/L appear to adversely affect the cysts. The alum dosages used for coagulation were always less than 25 mg/L.

Summing up these findings, it seemed reasonable to believe that the majority of cysts were still intact when reaching the coagulation and flocculation processes. Further, the large number of organisms unaccounted for as the flocculated water entered the filter were unlikely to have been lost because of attachment or entrapment on a surface.

In a final attempt to find a plausible explanation, a series of batch tests were performed. A known amount of cysts was added to a stainless steel pressure vessel containing 20 L of water that had been prefiltered through a 5 µm pore site membrane filter. The purpose of the filtration step was to remove diatoms which otherwise would have interfered with cyst enumeration, since the 20 L original sample at the end of the experiment was reduced to a final volume of 1 mL. The water was agitated with an electric mixer to ensure uniform distribution of the cysts. Then, the pH of the water was adjusted to a level such that optimum pH for coagulation and flocculation would be attained upon the addition of alum. Following the alum addition the water was flash mixed and flocculated for 20 minutes, and finally concentrated to a 1 mL volume using the cyst recovery technique described earlier.

Three series of these tests were conducted at different pressures and with alum dosages of 0, 4, 7, and 12 mg/L. In the first series, all 20 L samples were filtered at 10 psi positive pressure. For the second series the pressure was increased to 45 psi and finally, the samples were filtered at 80 psi.

As the results in Figure 45 indicate, the loss of cysts during the recovery increased with increasing pressure which could lead one to conclude that a growing number of cysts were forced through the filter membrane. This simple explanation, however, cannot account for the dramatic rise in the cyst loss experienced with increasing alum dosages. Neither did it seem likely that the organisms were sticking to the filter surface. After washing, several sections of each filter were examined microscopically without detecting any cysts.

A more probable theory is one which assumes that a significant number of the cysts, unaccounted for after concentrating the 20 L sample, were actually destroyed because of the physical stress on the organism during filtration. In addition, the

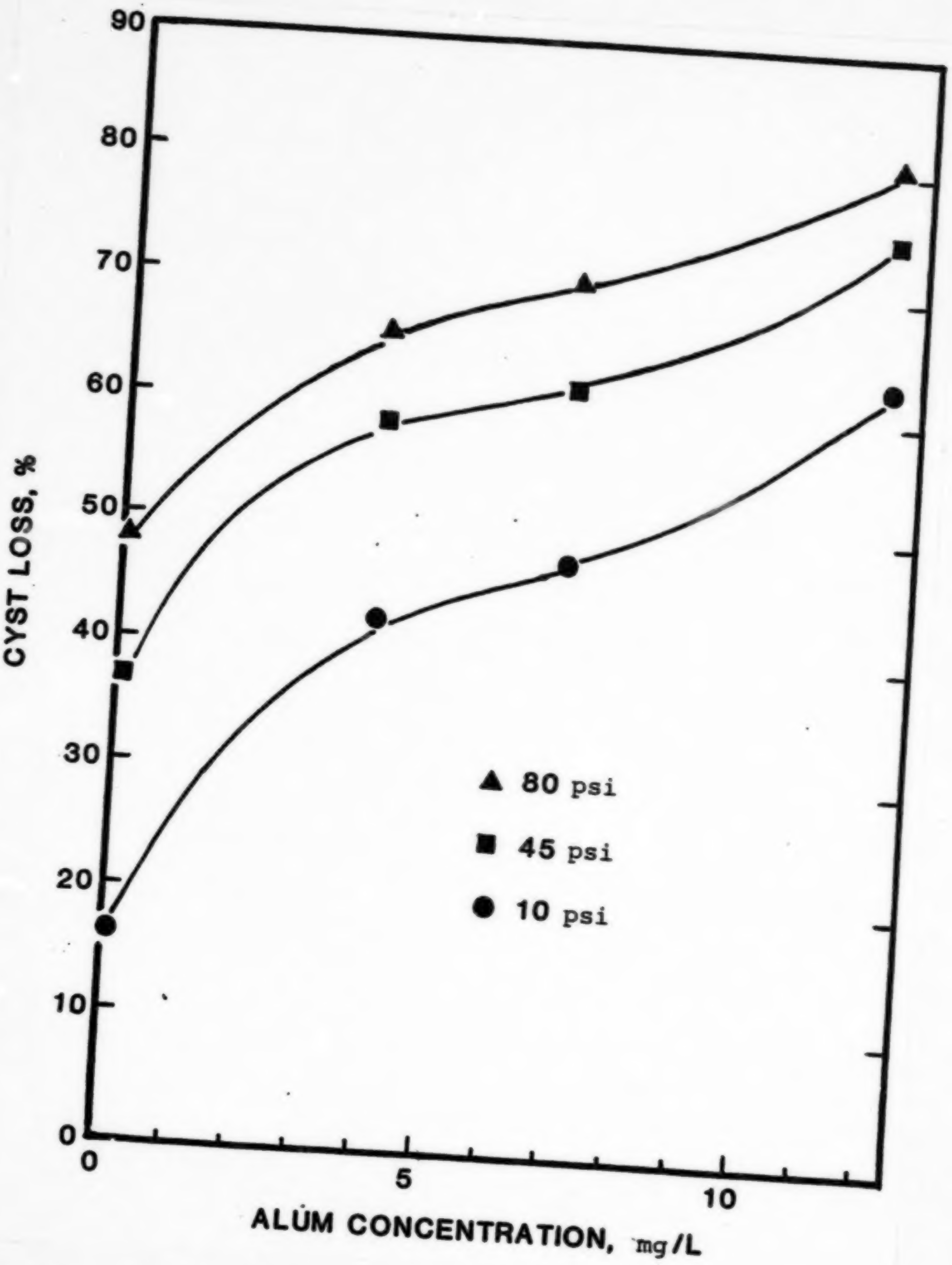


Figure 45. Effect of pressure and alum concentration on Giardia cyst losses.

presence of alum in the water weakens the cyst wall and makes it more susceptible to damage by physical forces. The observation of recognizable cyst fragments in some of the concentrated samples lends additional support to this hypothesis.

SECTION 7

QUALITY CONTROL

Throughout the body of the report, reference has been made to checks and procedures used to assure the validity of the data collected and to provide for reliable, reproducible test procedures.

During some periods of the research, extensive effort was expended on developing reliable, operable equipment, on assuring adequate test methods, and on using valid biological test procedures. The search for mechanisms to assure excystability of viable *Giardia* cysts was one such study area. When suitable methods for reliable excystability of *G. lamblia* could not be found, it was necessary to revert to the use of the surrogate, *G. muris*.

Suitable reference material for laboratory guidance on quality control is available in publications such as the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories (1972).

REFERENCES

Abbott laboratories. 1982. Schneierson's Atlas of Diagnostic Microbiology. Eighth Edition, edited by E. J. Battone, R. Girolami, and J. M. Stamm. 80 pp.

Baker, M. N. 1948. The Quest for Pure Water. Am. Water Works Assoc., Inc., New York. 527 pp.

Bingham, A. K., E. L. Jarroll, and E. A. Myer. 1979. Physical Factors of Excystation in Vitro and Excystation vs. Eosin Exclusion as Determinants of Viability. Exp. Parasitol. 47:1284-1291.

Blocker, J. M. 1929. The Purification of Water by Ultraviolet Radiation. JAWWA. 21:1361.

Britton, G., Y. Henis, and N. Lahav. 1972. Effect of Several Clay Minerals and Humic Acid on the Survival of Klebsiella aerogenes Exposed to Ultraviolet Irradiation. Appl. Microb. 23(5):870-874.

Buttolph, L. J., H. Haynes and I. Malelsky. 1953. Ultraviolet Product Sanitation. General Electric Bulletin LD-14.

Council on Physical Medicine Report. 1948. Acceptance of Ultraviolet Lamps for Disinfecting Purposes. JAMA. 137:1600.

DeWalle, F. B., J. Engeset, and W. Lawrence. 1983. Removal of Giardia lamblia Cysts by Drinking Water Treatment Plants. Grant. No. R80127, Municipal Environmental Research Laboratory. EPA-600/2-84-069. U.S. Environ. Prot. Agency, Cincinnati, Ohio. 112 pp.

Ellis, C. and A. A. Wells. 1941. The Chemical Action of Ultraviolet Rays. Revised Ed., by F. F. Heyroth. Reinhold Publishing Corp., New York. 961 pp.

Handbook for Analytical Quality control in Water and Wastewater Laboratories. 1972. U.S. Environ. Prot. Agency, Cincinnati, Ohio.

Hill, W. F., F. E. Hamblett, and E. W. Akin. 1967. Survival of Poliovirus in Flowing Turbid Seawater Treated with Ultraviolet Light. Appl. Microb. 15(3):533-536.

Hoather, R. C. 1960. Disinfection. J. Brit. Water Works Assoc. 42(348)535.

Hoff, J. C. 1979. Disinfection Resistance of Giardia Cysts: Origins of Current Concepts and Research in Progress. In: Waterborne Transmission of Giardiasis. W. Jakubowski and J. C. Hoff (eds.), EPA-600/9-79-001. pp. 231-239.

Hoff, J. C. and E. E. Geldreich. 1981. Comparison of the Biocidal Efficiency of Alternative Disinfectants, JAWWA, 73:40-44.

Jagger, J. 1967. Introduction to Research in Ultraviolet Photobiology. Englewood Cliffs. Prentice-Hall. 164 pp.

Johnson, J. D., R. G. Qualls, K. H. Aldrich, and M. P. Flynn. 1982. Ultraviolet Disinfection of Secondary Effluents, Dose Measurement and Filtration Effects. Delivered to 2nd National Symposium of Municipal Wastewater Disinfection, Orlando, Florida. January 27, 1982.

Juranek, D. 1979. Waterborne Giardiasis (Summary of Recent Epidemiologic Investigations and Assessment of Methodology). In: Waterborne Transmission of Giardiasis. W. Jakubowski and J. C. Hoff (eds.), EPA-600/9-79-001. pp. 150-163.

Kasprzak, W. and A. C. Majewska. 1983. Infectivity of Giardia sp. Cysts in Relation to Eosin Exclusion and Excystation in Vitro. Tropenmed. Parasit. 34:70-72.

Koller, L. R. 1965. Ultraviolet Radiation. 2nd Ed. John Wiley & Sons, Inc., New York. 312 pp.

Lawrence, C. A. and S. S. Block. 1968. Disinfectants, Sterilization and Preservation. Lea & Febiger, Philadelphia. 808 pp.

Leighton, T. J. and R. H. Doi. 1971. The Stability of Messenger Ribonucleic Acid during Sporulation in Bacillus subtilis. J. Biol. Chem. 246(10):3189-3195.

Lengyel, B. A. 1971. Lasers. 2nd Ed. Wiley-Interscience, New York. 386 pp.

Levenspiel, O. 1972. Chemical Reaction Engineering, 2nd Ed. John Wiley and Sons, Inc. 578 pp.

Lippy, E. C. and S. C. Waltrip. 1984. Waterborne Disease Outbreaks - 1946-1980: A Thirty-Five Year Perspective. JAWWA. 76(2):61-67.

Logsdon, G. S., J. M. Symons, R. L. Hoye, Jr., and M. M. Arozarena. 1981. Alternative Filtration Methods for Removal of Giardia Cysts and Cyst Models. JAWWA. 73:111-118.

Luckiesh, M. and L. L. Holladay. 1944. Disinfecting Water by Means of Germicidal Lamps. General Electric Review. 47:45.

Meckes, M. C. 1982. Effects of UV Light Disinfection on Antibiotic-Resistant Coliforms in Wastewater Effluents. Applied and Environ. Microbiol. 43:371-377.

Oda, A. 1969. Ultraviolet Disinfection of Potable Water Supplies. Division of Research, Ontario Water Resources Commission, R.P. 2012. 48 pp.

Petrasek, A. C., Jr., H. W. Wolf, S. E. Esmond, and D. C. Andrews. 1980. Ultraviolet Disinfection of Municipal Wastewater Effluent. EPA 600/2-80-102, U.S. Environ. Prot. Agency, Cincinnati, Ohio. 262 pp.

Phillips, R. 1983. Sources and Applications of Ultraviolet Radiations. Academic Press, Inc., New York. 434 pp.

Qualls, R. G., M. P. Flynn, and J. D. Johnson. 1983. The Role of Suspended Particles in Ultraviolet Disinfection. JWPCF. 55:1280-1285.

Qualls, R. G. and J. D. Johnson. 1983. Bioassay and Measurement of Dose in Ultraviolet Disinfection. Appl. Environ. Microbiol. 45:872-877.

Reddish, G. F. 1957. Antiseptics, Disinfectants, Fungicides and Sterilization. 2nd Ed. Lea & Febiger, Philadelphia. 975 pp.

Rice, E. W. and J. C. Hoff. 1982. Inactivation of Giardia lamblia Cysts by Ultraviolet Irradiation. Appl. Environ. Microbiol. 42:546-547.

Rice, E. W. and F. W. Schaefer. 1981. Improved In Vitro Excystation Procedure for Giardia lamblia Cysts. J. Clin. Microbiol. 14:709-710.

Rice, E. W., J. C. Hoff, and F. W. Schaefer. 1982. Inactivation of Giardia Cysts by Chlorine. Appl. Environ. Microbiol. 43:250-251.

Roeber, J. A. and F. M. Hoot. 1975. Ultraviolet Disinfection of Activated Sludge Effluent Discharging to Shellfish Water. Environmental Protection Technology Series, EPA-WPRD 139-01-68.

Schaefer, F. W., III, E. Rice, and J. C. Hoff. 1980. *In vitro* Excystation of *Giardia muris* Cysts. Proceedings of the 55th Annual Meeting of the American Society of Parasitologists. Aug. 4-8, 1980. Univ. of California, Berkeley. Abstract 90. p. 51.

Schenck, G. O. 1979. Possibilities of Disinfecting Water by Ultraviolet Irradiation. Institut fur Strahlenchemie im Max-Planck-Institut fur Kohlenforschung, Mulheim a.d. Ruhr. Parfumerie u. Kosemtik.

Sepp, E. and P. Bao. 1981. Design Optimization of the Chlorination Process. Vol. I. Comparison of Optimized Pilot System with Existing Full-Scale Systems. EPA-600/2-81-167. U.S. Environ. Prot. Agency, Cincinnati, Ohio. 124 pp.

Severin, B. F., M. T. Suidan, and R. S. Engelbrecht. 1983. Kinetic Modeling of UV Disinfection of Water. Water Res. 17:(11):1669-1678.

Severin, B. F., M. T. Suidan, B. E. Rittmann, and R. S. Engelbrecht. 1984. Inactivation Kinetics in a Flow-Through UV Reactor. JWPCF. 56(2):164-169.

Sheffield, H. G. and B. Bjorvatn. 1977. Ultrastructure of the cyst of *Giardia lamblia*. Amer. J. Trop. Med. Hyg. 26:23-30.

Standard Methods for the Examination of Water and Wastewater. 1975. 14th Edition. APHA-AWWA-WPCF. 1193 pp.

Standard Methods for the Examination of Water and Wastewater. 1980. 15th Edition. APHA-AWWA-WPCF. 1134 pp.

Venosa, A. D. and E. J. Opatken. 1979. Ozone Disinfection - State of the Art. Proc. Pre-Conf. Workshop on Wastewater Disinfection. WPCF.

Walden, A. E. and S. T. Powell. 1911. Sterilization of Water by Ultraviolet Rays. Proc., AWWA. p. 341.

Wangsuphachart, S. and D. A. Carlson. 1984. Excystation of *Giardia muris* Cysts as Surrogate Indicator Organisms of *Giardia lamblia*, a Human Infecting Species. Unpublished report.

Yip, R. W. and D. E. Konasewich. 1972. Ultraviolet Sterilization of Water -- Its Potential and Limitations. Water and Pollu. Control. 110(6):14-15, 17-18.